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Migratory flight potential and the role of juvenile hormone in flight regulation in the western corn rootworm

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**MIGRATORY FLIGHT POTENTIAL AND THE ROLE OF JUVENILE HORMONE
IN FLIGHT REGULATION IN THE WESTERN CORN ROOTWORM**

Iowa State University

Ph.D. 1986

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**Migratory flight potential
and the role of juvenile hormone in flight regulation
in the western corn rootworm**

by

Susan Anne Orkins Coats

**A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY**

Major: Entomology

Approved:

Signatures have been redacted for privacy.

**Iowa State University
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INTRODUCTION

Background Information

The amount of literature published on the western corn rootworm (WCR), Diabrotica virgifera virgifera Leconte, attests to the economic importance of this species (Luckmann et al. 1974, Irwin 1977). It is an important corn pest in the Midwest, with both larvae and adults inflicting major damage on the corn plant throughout the growing season (Chiang 1965, 1973).

Ninety percent of all corn planted after corn in Iowa during 1985 was treated with insecticides for corn rootworm control (Iowa State Extension Service, Ames, Iowa, unpublished). The conventional, often prophylactic, insecticide applications against larvae in the spring and adults in the summer has resulted in corn being ranked as number one in the U. S. in the total area treated and second in insecticide usage (Eichers et al. 1976). In the 1980s, over 700 million pounds of insecticides per year are being used (R. L. Metcalf 1986), while the loss of potential yields in crops to insects is equivalent to the losses reported thirty years ago (Guthrie and Perry 1980).

One reason for such abundant use is due to the inefficiency of mass application. For example, for the WCR there is a $1 \times 10^7\%$ inefficiency rating based on application of 0.5 μg carbamate per beetle, an economic threshold for adult damage of five beetles per corn plant, and 150,000 corn plants per acre (Metcalf 1986). Additionally, insecticide usage has resulted in a host of problems. There are over 500 resistant

species of insects in the 1980s (compared to zero in 1910) (Metcalf 1986). Cross resistance occurs across classes of compounds for some pests, with increasingly shorter amounts of time being required for resistance to develop (Metcalf 1986). Biomagnification of chemicals through the food chain has also resulted. The DDT metabolite, DDE, has been magnified 200,000 times, causing major mortality in carnivorous birds through egg shell thinning (Guthrie and Perry 1980). Application of .01% concentration of dieldrin on soybeans used for feed resulted in the destruction of chickens which concentrated the compound 50 times (Metcalf 1986).

Enhanced biodegradation of insecticides due to alterations in the numbers and composition of species of microflora (Kaufman and Edwards 1983) has further restricted the usefulness of many insecticides. Researchers are just beginning to recognize the complex of interactions between such microflora and insecticides, herbicides, and fertilizers (Kaufman and Edwards 1983).

Alternative strategies to control insects such as the WCR are necessary. Before effective control is possible, it is essential we understand why the WCR is so successful in its invasion of corn. The WCR is basically a monophagous feeder limited to maize (Painter 1951, Smith 1966). Reliance on a single crop for survival has required the WCR to adapt its biology to insure contact with its food source. One adaptation is the synchronization of its life cycle with the corn crop, another is its dispersal capabilities through flight. Research to date (Godfrey and Turpin 1983, Hill and Mayo 1980, VanWoerkom et al. 1983)

supports the hypothesis that the WCR has extensive flight capabilities which enable it to disperse rapidly and reproductive capabilities which permit rapid colonization of all new acceptable habitats (Hill 1975). A key to management, therefore, may be the control of the long- and short-range dispersal of this pest and/or its reproduction.

The potential for regulating pest species through interruption of biological processes with application of synthetic juvenile hormone (JH) agonists and antagonists is one possible future control measure. Theoretical management capabilities could result from crop rotation to disrupt food supply coupled with synthetic JH application to effect premature ovarian development; this could result in the production of non-viable eggs and premature flight muscle histolysis, which would limit dispersal flights to other fields. It is imperative, therefore, that the exact details of the role JH plays in the developmental physiology of flight and vitellogenesis be obtained for this species. With this knowledge, the regulation of the WCR will be plausible with JH. Application of JH mimic (JHM) or JH inhibitor (AJH) to critical ages during the life of mature adult females could derange normal biological processes such as reproduction and flight. Disruption of these biological processes requires specific knowledge of which ages would be susceptible, and what rates of application are necessary for desired effects.

Long-range improvements in food production may result through lowered production costs and elimination of resistance and resurgence

problems. JHM or AJH would be species specific, environmentally safe pest control compounds (Sláma et al. 1974).

Biology of Western Corn Rootworm

The WCR is univoltine. Eggs are laid during the late summer until the first killing freeze (Ball 1957). These eggs winter in the soil, hatching the following spring during the months of May and June at most northern latitudes (Chiang 1965, 1973). Adults begin emerging in the middle of July with peak emergences by the middle of August (Ortman and Fitzgerald 1964). Males emerge a few days ahead of females. Females usually mate only once, while males may mate with several females (Lew and Ball 1979).

There is a preovipositional period of 12-14 days in adult females (Short 1970). Five ovarian stages have been reported (Short 1970), with stage 4 ovaries associated with the beginning of oviposition. The female lays an average of 1100 eggs over about two months (Branson and Johnson 1973, Short and Hill 1972).

The female reproductive system has two laterally placed ovarian lobes, with an average of 100 ovarioles per insect (Short 1970). A terminal filament, a biologically active germarium, and vitellarium make up each ovariole. Terminal filaments from ovarioles join into a common median filament from each ovary and attach the ovaries to the body wall. The ovarioles are considered to be the meroistic type. The egg calyx enlarges into the lateral oviducts. These join to form a common oviduct, which opens into the bursa copulatrix. The spermatheca lies posterior of the bursa copulatrix.

Juvenile Hormone

Although JH is named for its essential role in the development of immature insects, it elicits dramatic and significant effects in adult insects. In 1936, Wigglesworth described a reproductive effect attributable to the corpora allata. Since that time there has been considerable progress in understanding the importance of JH in the adult insect. Vitellogenin synthesis in the fat body is governed by JH (Chen and Wyatt 1981, Englemann 1983, Berovsky et al. 1985). Additionally, JH affects the maturation of the ovaries (Koeppel 1981, Davey 1983, Renucci et al. 1984). Oöcytes in vitellogenic ovaries may stimulate an increase in JH synthesis (Rankin and Stay 1984). Sterility can be imparted to adult insects by treatment with JHM (Sehnal 1983). Development of the colleterial gland is regulated through JH (Willis and Brunet 1966, Pau 1981). The follicle cells are affected by JH prior to vitellogenin deposition (Davey 1981). The production of sex pheromone depends on JH involvement in some insects (Blomquist and Dillwith 1983).

In addition to the effects on reproduction, the important influence of JH on other functions in adult beetles has also been recognized. Diapause is controlled in part by JH in the haemolymph (deWilde 1983). Identification of the role of JH as a trigger of migratory flight (Caldwell and Rankin 1972; Goldsworthy et al. 1972, 1973; Rankin 1974; Mayer and Candy 1969) presents researchers with another tool for testing and influencing the migratory behavior in a species. JH and AJH have been extensively tested on the convergent lady beetle by Rankin and Rankin (1980a,b). Methoprene, a JHM, significantly increased

migratory flight in young adults while precocene II, an AJH, significantly inhibited such flight. Data have also revealed that JH may induce flight muscle histolysis, causing flight inhibition in older females of reproductive age (Nair and Prabhu 1985, Borden and Slater 1968), while inducing egg maturation and deposition.

The specific mechanism of JH in the induction of such observable effects on flight are possibly associated with alterations in DNA synthesis, RNA synthesis, and protein (vitellogenin synthesis in the fat body). The alterations increase in the preovipositional female (Hagedorn and Kunkel 1979).

Migration

Although the idea of migration has been widely accepted, whether or not any one particular species of insect truly migrates or is passively disseminated on the winds has become a matter of conjecture. The workers who have addressed this question have provided a variety of answers. Some researchers have considered true migration to be a long-range flight to and from a habitat as in the Monarch butterfly, Danaus plexipus (Heape 1931, Brower 1961). Given the short longevity of many insects, the return flight (re-migration) may be made by offspring of the original fliers. In such cases, the species may still be considered migratory (Allee et al. 1949, Williams 1930, Nielsen 1961). Insects drifting on the wind were considered accidental dispersants (Lawson et al. 1951, Glick 1957, Carter 1961). Disagreement with the accidental dispersal of insects occurred because it was noted that insects are not passively transported by the wind; rather, they undergo considerable

effort to reach the heights where prevailing winds occur and must continually persist in locomotory movements while ignoring factors that might stimulate landing, such as food or mates (Kennedy 1951). The criterion for migration was subsequently defined as: persistent locomotion, with migrants distinguished from non-migrants by their "transient accentuation of locomotor functions with a depression of vegetative functions, such that the insects now travel, by what method being a secondary matter" (Kennedy and Stroyan 1959). Migratory flight was also considered as "non-appentential" or "a special flight which serves no physiological need, whatever teleological end it may serve" (Provost 1952). There is a general consensus among authors (Southwood 1962, Corbet 1962) that "undistractedness and persistence are criteria of migratory flight" (Johnson 1969).

The degree of persistence that marks a flight as migratory has been defined as flights of 30 minutes or more (Dingle 1972, 1978). It was observed that females which flew for 30 min would continue flying for much longer periods. Also, a flight of 30 min allows the insect to reach the level of prevailing winds, which accounts for the large displacement of many migrants (Johnson 1969).

The complex relationship of migration to diapause has also been defined. The process of migration is a part of the "diapause syndrome" because it is associated with the same physiological changes in the insect; these changes, in turn, are precipitated by the same environmental stimuli (Tauber et al. 1984). Additionally, migratory

flight may be further classified by the conditions which induce its occurrence and/or the behavior of the insect following its completion; seasonal and aseasonal migration are such delineations (Tauber et al. 1984).

This research was undertaken to define migratory flight potential in the WCR and to evaluate the catalyst (or inhibitor) of such flight through the modification of juvenile hormone levels. A correlation of ovarian maturation with flight activity was also undertaken, permitting an analysis of the temporal relationship between flight and reproduction. Compilation of all such data may permit the flight capabilities of the female WCR to be objectively appraised. Quantification of short- and long-range dispersal would provide one explanation for the success of this major corn pest.

Explanation of Dissertation Format

This document contains two manuscripts written for publication in scientific journals. Research for these studies was performed from 1982 through 1985 at Iowa State University. All flight mill testing was done in the laboratory of Dr. Wayne Rowley, who, along with John L. Clarke, III, instructed me on the use of the equipment. The electron micrographs were taken during the fall of 1985 under the direction of Dr. Harry Horner. This research and the writing of these manuscripts was co-supervised by Drs. Jon Tollefson and John Mutchmor, who share with me co-authorship of these publications.

**A STUDY OF MIGRATORY FLIGHT IN THE WESTERN CORN ROOTWORM
(COLEOPTERA: CHRYSOMELIDAE)**

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SECTION 1. A STUDY OF MIGRATORY FLIGHT IN THE WESTERN CORN ROOTWORM
(COLEOPTERA: CHRYSOMELIDAE)

Introduction

Archeological and biosystematic records indicate the Western corn rootworm (WCR), Diabrotica virgifera virgifera, is tropical in origin (Krysan 1982, Krysan et al. 1982). With the cropping of corn, the WCR probably has immigrated to the North American continent during the past 1,000 years (Branson and Krysan 1981). It was collected in Kansas in 1867 (LeConte 1868-1869, who named the species). The WCR was first identified as a pest of corn in Colorado in 1909 (Gillette 1912). By the 1940s, this insect emerged as a major corn pest in Nebraska and Kansas (Ball 1957). Now in the 1980s, the WCR has expanded its range to include all the territory from the Rocky Mountains to the eastern seaboard (personal communication, J. L. Krysan, USDA, Yakima Agr. Res. Lab, Yakima, Washington).

The WCR is univoltine and basically a monophagous insect. It usually lays its eggs in corn, and larvae can only survive in appreciable numbers in this crop (Painter 1951, Smith 1966). In order to be a significant economic pest of corn, the rootworm must be able to rapidly re-colonize cornfields that are planted after other crops (Ball 1957, Chiang 1965, 1973, Krysan 1982).

During the 1985 growing season in Iowa there were a total of 4,785,000 acres of corn planted after corn (continuous corn). Corn

planted for only two consecutive years accounted for a large percentage of these acres. Insecticide treatment to control rootworm infestation was applied to 90% of this acreage (Iowa State Extension Service, 1985, Ames, Iowa, unpublished data).

The necessity for these control practices demonstrates the ability of the WCR to rapidly reinfest all suitable habitats, which, in turn, illustrates its great mobility. With the vast expanse of corn available across the midwest, beetles disperse rapidly from their emergence sites (donor fields) to neighboring first-year corn (receptor fields). Since continuous corn may senesce sooner than rotated corn fields, especially under stress conditions, movement of adult, mated, preovipositional females to the still-succulent growth of rotated corn occurs from donor to receptor fields throughout the season (Hill and Mayo 1980, Godfrey and Turpin 1983).

The dispersal of this insect over such a large area and between fields within that area in such short periods of time indicates a sustained flight capability in the WCR. Migration has been implicated in the rapid coverage of area by the WCR (Hills et al. 1972, Owens et al. 1974, Rasmussen and Chiang 1967). Complementary evidence of mass movement comes from studies of resistance of WCR beetles to cyclodiene insecticides. This resistance rapidly spread eastward over Nebraska and neighboring states in the period 1959-1961 (Ball and Weekman 1962, 1963). Similar areas of resistance were still found in 1976 and 1981 (Krysan et al. 1982). Isozyme studies on beetles within and outside the area of recent range expansion demonstrate little inter- and

intra-population variability in WCR (Krysan et al. 1982, McDonald et al. 1982, 1985). This indicates sustained flight capability in the WCR according to theories of migration and differentiation of populations (Wade 1982).

Other evidence of non-appetential flight includes observations (M. K. Bergman and T. T. Turpin, Department of Entomology, Purdue University, West Lafayette, Indiana) of large numbers of adult WCR beetles on the shores of Lake Michigan in the fall. This phenomenon is understandable if WCR beetles were flying on prevailing westerlies and were caught in a rapid downdraft when confronted with the temperature effects of the winds off the lake. A mass-landing of this kind was documented for migrants (Rainey 1951, Glick 1957), and is illustrated by the convergent lady beetle, Hippodamia convergens and thirteen spotted lady beetle, Hippodamia tredecimpunctata, which display similar mass landings on the shores of lakes in the upper Midwest (Lee 1980). Recent information indicates movement of 70 miles by WCR founder populations (Balsbaugh 1980).

Documentation of persistent flight has proved difficult, with techniques of mark and release or tethered flight yielding disappointing results for many small species. New equipment such as the flight mill and microcomputer accessories allow precise measurement of sustained flight activity of the WCR (Rowley et al. 1968).

Materials and Methods

Adult WCR were collected at the same time daily from emergence cages during the summer of 1984 and maintained in 30.5 cm³ screened cages in a greenhouse. Windows were opened and artificial light was not provided, thus approximating natural conditions of temperature and photoperiod. Lettuce, fresh corn ears and tassels, as well as artificial diet (Branson et al. 1975), were supplied to the beetles daily.

All flight testing was done on a flight mill system (Rowley et al. 1968). Each flight mill was covered by a plastic hood to prevent drafts that might confound activity and then placed in a walk-in incubator maintained at a constant temperature of $25.6 \pm 1^{\circ}\text{C}$ and $80 \pm 5\%$ R.H. A 28-V lamp on each of the twelve flight-mill arms provided the only light. These conditions simulated twilight. For each flight, beginning and ending times, distance, and speed were recorded with a Pet Commodore 2400 microcomputer (Clarke et al. 1984).

All females were confirmed to be mated either by dissections to determine ovarian stage (Short and Hill 1972), smear samples for sperm, or holding flown females in isolation and determining the viability of their eggs. Mated female beetles (n=183) were mounted by the dorsum of the pronotum to the flight mill arms with a temperature-indicating wax and allowed to fly for a period of 24 h to determine periodicity. During this 24-h period, they always remained suspended from the flight mill arms. Flights of less than one meter were not recorded by the computer. Each female was examined for only one 24 h period. Females

tested ranged in age from 2-15 days post emergence. Food, consisting of corn ears, and males contained in 6.35 cm diameter screened cages were placed on the floor of each hooded flight mill.

Two means were calculated for each female: one for all trivial flights and one for all sustained flights made during the 24 h test period. The means for each flight type for all females were combined for statistical analysis (Snedecor and Cochran 1967). Speed was a calculated variable, equal to distance divided by time. The program used for processing flight information produced this value as part of its regular output.

Results

Flight duration

A total of 183 mated female WCR adults was flown over a period of 4 weeks, starting 27 July. These beetles flew a total of 3,838 flights. Sustained flights have been defined as those lasting 30 min or more in length (Dingle 1965, Rankin and Rankin 1980). When this 30-min limit is applied to the data acquired on 6-day-old beetles (Fig. 1), it seems to be a valid determinant and is representative of all the ages tested. There are two types of flights: trivial and sustained. The majority of flights lasted 1 min or less or from 1 to 17 min and are considered trivial flights (Table 1); sustained flights were those lasting considerably longer, from 3/4 h to almost 4 h (Table 2).

When the 30-min criterion is applied to all ages tested, it clearly differentiates between the two types of flights (Fig. 2). Twenty-eight

females (or 15%) flew 127 sustained flights, ranging from 42 to 230 min. The average flight time for these sustained fliers was 71.8 min, whereas average trivial flight length was 3.1 min.

Flight-age relationship

The sustained flights were made by females ranging in age from 2 to 9 days posteclosion. Fig. 3 represents the percentage of flights by age of total flights. No sustained flights were attempted after the ninth day, but trivial flights occurred through day 15, the last age tested. The greatest number of sustained and trivial flights was made by the 5- and 6-day-old beetles.

Flight distance

Distance flown (Fig. 4) is a corollary to time flown. The longest distance in one flight was 24 km. The longest distance for any 24-h period by one beetle was 39.6 km. The average distance for sustained flights was more than 5-fold greater than the average distance for trivial flights. There was an increase in distance through day 6, then a general decline for both types of flights.

Flight speed

The type of flight influenced flight speed. There was a 3-fold difference in velocity between the two types of flights, with sustained fliers averaging almost 50 m/min, and trivial fliers averaging 16 m/min. Speed remained fairly constant throughout life for the range of ages tested. The greatest speed attained by a sustained flier was 10 km/h, compared with 2.1 km/h for a trivial flier.

Flight periodicity

There was a definite flight periodicity (Fig. 5). Sustained flights occurred only during the early morning and evening, with most flights occurring from 1800 to 2200 hours. Conversely, trivial flights occurred during any part of the day, with a propensity for activity between the hours of 1800 and 2400.

Ovarial maturation

Ovaries of all sustained fliers were dissected. Ovarial maturation was classified as either stage one (age 2 to 4) or stage two (age 5-9) of development (Short 1970). The trivial fliers were only determined to have mated, and ovarian development according to stage was not attempted.

Discussion and Conclusions

Classification of sustained flight activity as migration

Migratory flight in insects has been delineated as sustained, non-appetential flight activity which lasts 30 minutes or more (Dingle 1965). WCR female beetles can fly continuously for as long as 4 h, covering about 24 km in one flight. Projecting the mean distance covered per day (35 km) for sustained fliers over only the first 6 days of their life suggests that beetles are capable of wind-unassisted flights covering well over 200 km.

In this study, the life stage during these long-range flights is preovipositional (stage 1 or 2 ovarian classification per Short 1970). This degree of ovarian development is a common characteristic of

migratory females (Tauber et al. 1984, Rankin and Rankin 1980, Johnson 1969); it enables rapid deployment of colonizers when a new destination is reached (Dingle 1966, 1978).

The sustained flights by the WCR occurred during the early morning or evening hours. This crepuscular flight pattern is supported by field sampling data (VanWoerkom et al. 1980, 1983) and research by others (Cates 1968, Kaufman 1966, Witkowski et al. 1975). Flights during these times occur in periods of reduced wind, permitting greater self-control by beetles over flight direction (VanWoerkom et al. 1983). Without the hindrance of surface winds, female WCR, like other insects, can attain the elevation necessary for transport on prevailing winds, i.e., migrate (Johnson 1969).

The sex ratio of WCR in cornfields becomes increasingly skewed toward females as height above ground increases: from 1:1 (F:M) at three and four m to 5:1 (F:M) at six and seven m (Witkowski et al. 1975, VanWoerkom et al. 1983). These data are consistent with the upward movement toward prevailing winds by migratory females.

Preovipositional female WCR make sustained flights of over 30 minutes in length during morning and evening hours on the flight mill. Field samples show that preovipositional WCR females are present at considerable heights above ground during these same hours (VanWoerkom et al. 1983). Circumstantial evidence (presented in the Introduction) supports the conclusion that flight capability is the mechanism by which the WCR has expanded its range to include over two-thirds of the United

States in only 40 years. Sustained flight activity in the WCR is therefore classified as migration.

Classification of migration as aseasonal

The WCR is a major corn pest in the cornbelt because it has a very successful life history strategy. Synchronization of its development with corn production through egg diapause insures its survival in a seasonal climate with harsh environmental conditions. Exploitation of all available habitats is accomplished through the use of its considerable flight capabilities.

The length of diapause in the WCR is negatively correlated with higher latitudes. The genetic plasticity of the diapause-duration trait is illustrated by the development of a non-diapausing strain in the laboratory (Branson 1976). This evidence suggests that natural selection maintains the shortest diapause that adjusts the insect's life cycle to the growing season (Krysan 1982).

The WCR must find new hosts annually since its host plant (corn) may or may not be in the same field from which it emerged, due to crop rotation. The WCR deals with this problem by investing "a small proportion of the reproductive stage of the population in migratory flight to new areas (which) increases the chance for survival if the old environment is eliminated through catastrophic events" (Dingle 1966, 1978; Kennedy 1961). Additionally, migration also allows a species to expand its numbers over a greater range and ensure survival of the species as a whole (Dingle 1978).

Both trivial and sustained flight activities occur in laboratory-reared females throughout the year and are not regulated by photoperiod. The major function of these flights is invasion of either near or distant host habitats, and do not occur as the result of seasonal deterioration of habitats. For sustained fliers, there is only a short-term postponement in reproduction, with feeding and reproduction resumed after migratory flights have occurred.

Since migratory flight complements diapause as an adaptation to a spatially heterogeneous environment, is hormonally influenced (Coats et al., Section 2 herein), is followed by feeding and reproduction, and occurs as a method for dispersal to new habitats, this type of migratory flight should be classified as aseasonal migration (Tauber et al. 1984).

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Table 1. Means of trivial flights according to age of 183 adult
mated ♀♀

Age (d)	n ^a	\bar{X} Flights/♀ ^b	\bar{X} Time \pm S.E. ^c (min)	\bar{X} Distance \pm S.E. ^c (meters)
2	17	20	1.65 \pm .04	38.0 \pm 2.8
4	13	11	2.30 \pm .32	46.0 \pm 1.8
5	23	22	5.00 \pm .18	145.0 \pm 4.7
6	25	34	6.50 \pm .22	202.1 \pm 13.4
7	17	25	2.00 \pm .01	60.5 \pm .06
8	15	26	3.50 \pm .05	60.0 \pm 1.2
9	25	21	3.30 \pm .02	66.1 \pm 3.1
10	14	20	3.40 \pm .03	44.3 \pm 0.9
11	15	7	1.70 \pm .53	27.2 \pm 1.1
13	12	6	1.80 \pm .31	25.2 \pm 2.4
15	7	11	2.80 \pm .11	31.0 \pm 1.7

^aNumber of ♀♀ = n for each age tested in days (d).

^bTotal number of flights = 3,711.

^cStandard errors (S.E.) are given for each mean of time and distance.

Table 2. Means of migratory flights according to age of 28 adult
mated ♀♀

Age (d)	n ^a	\bar{X} Flights/♀ ^b	\bar{X} Time \pm S.E. ^c (min)	\bar{X} Distance \pm S.E. ^c (meters)
2	3	5	61.5 \pm 8.3	2583.0 \pm 86.9
4	2	4	80.7 \pm 11.7	2824.5 \pm 93.2
5	8	5	79.6 \pm 3.4	3582.1 \pm 54.3
6	7	5	108.9 \pm 12.6	7296.3 \pm 68.4
7	3	2	56.7 \pm 8.5	3288.9 \pm 22.3
8	2	2	59.4 \pm 7.8	2970.0 \pm 14.3
9	3	5	56.1 \pm 9.2	2692.8 \pm 18.7

^aNumber of ♀♀ = n for each age tested in days (d).

^bTotal number of flights = 127.

^cStandard errors (S.E.) are given for each mean of time and distance.

Fig. 1. Flight times of 23 adult mated ♀♀, 6 days old. Note the 30-min limit that separates trivial and sustained flights.
Total flights = 885

Flight Times vs Time of Day for 6 Day Old Females

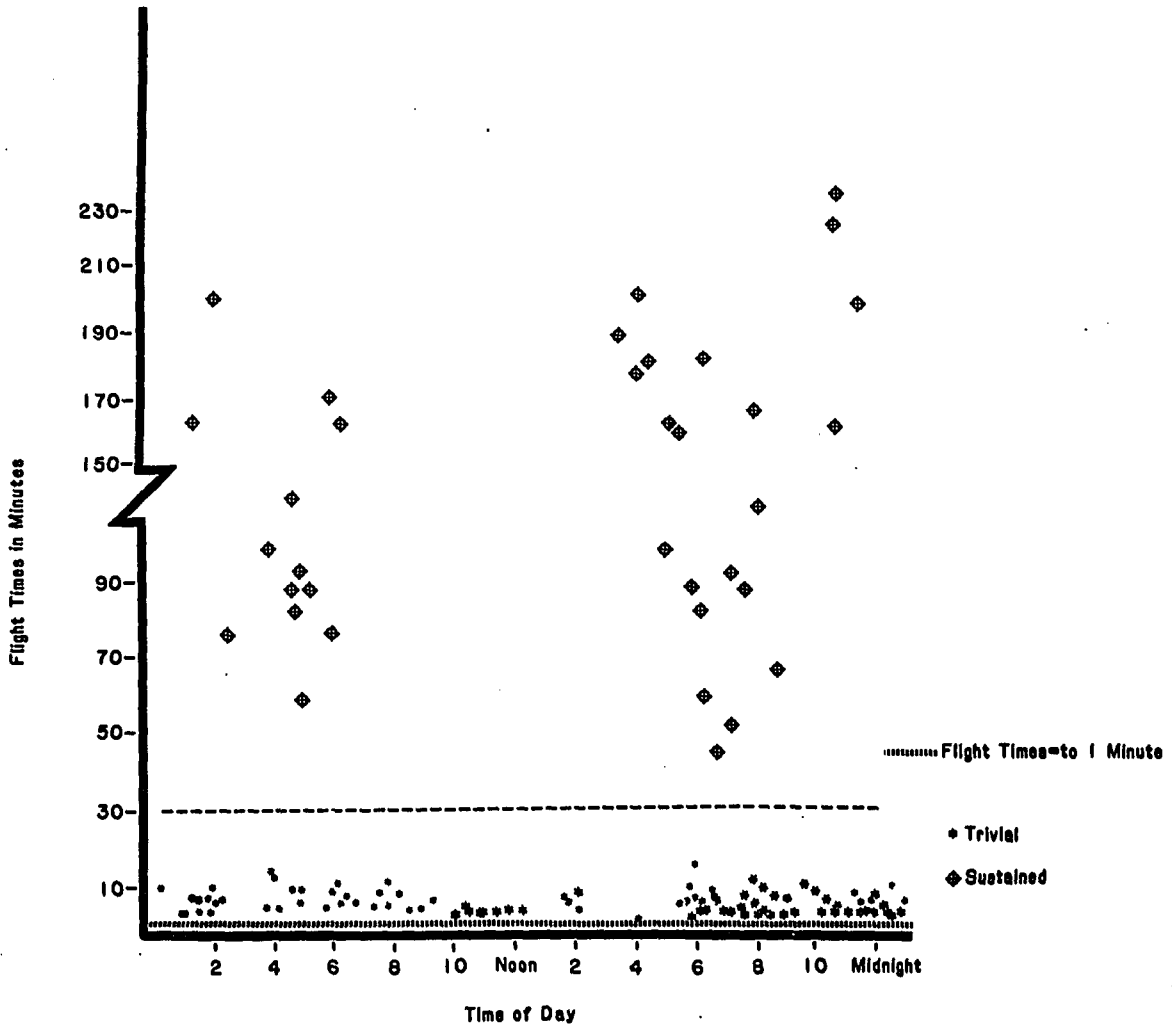


Fig. 2. Flight duration of all 183 ♀♀ tested. Total trivial flights = 3711, total sustained flights = 127. Means are calculated separately for each type of flight and for each ♀. Each bar represents the mean time flown by all adult ♀♀ of the same age. Trivial and sustained flights were determined by the 30-min limit and the means are plotted separately

Flight Times vs Age

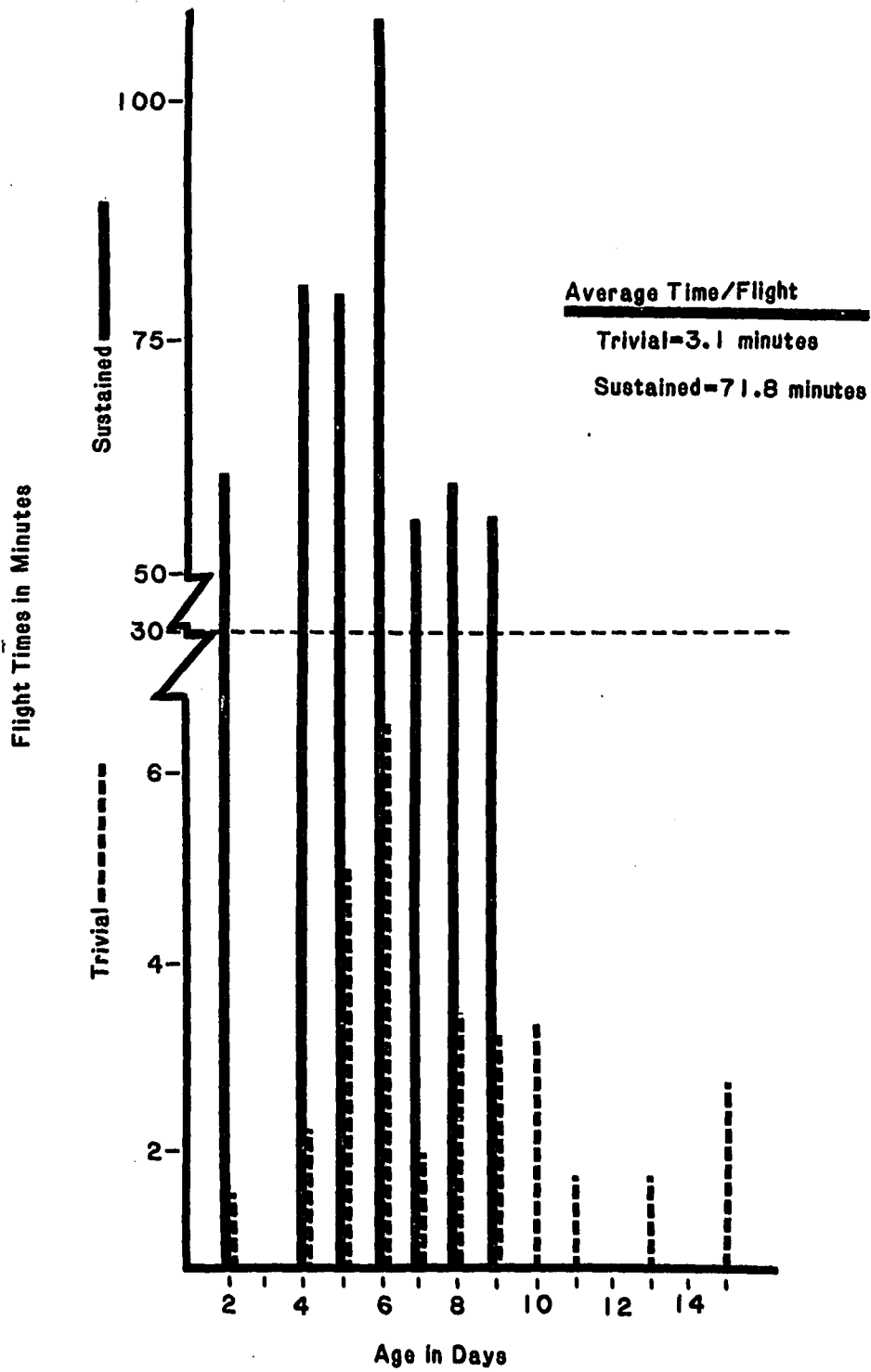


Fig. 3. Flight-age relationship. Each bar represents the percentage of the total trivial or sustained flights made by each age of ♀

Percentage of Flights by Age

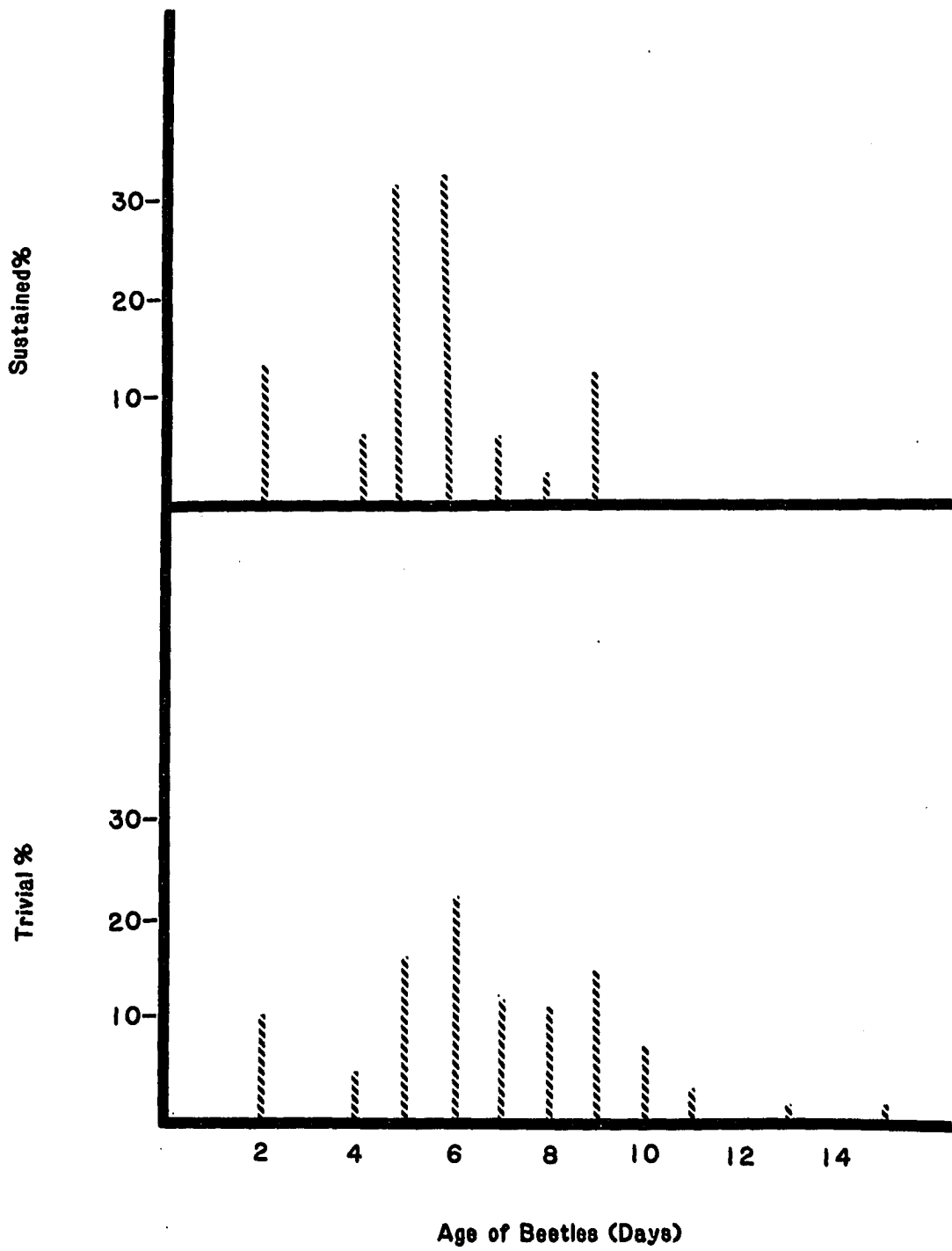


Fig. 4 Flight distance. Each point represents the mean distance flown by ♀♀ of the same age. Sustained distance averaged 5.3 x trivial distance

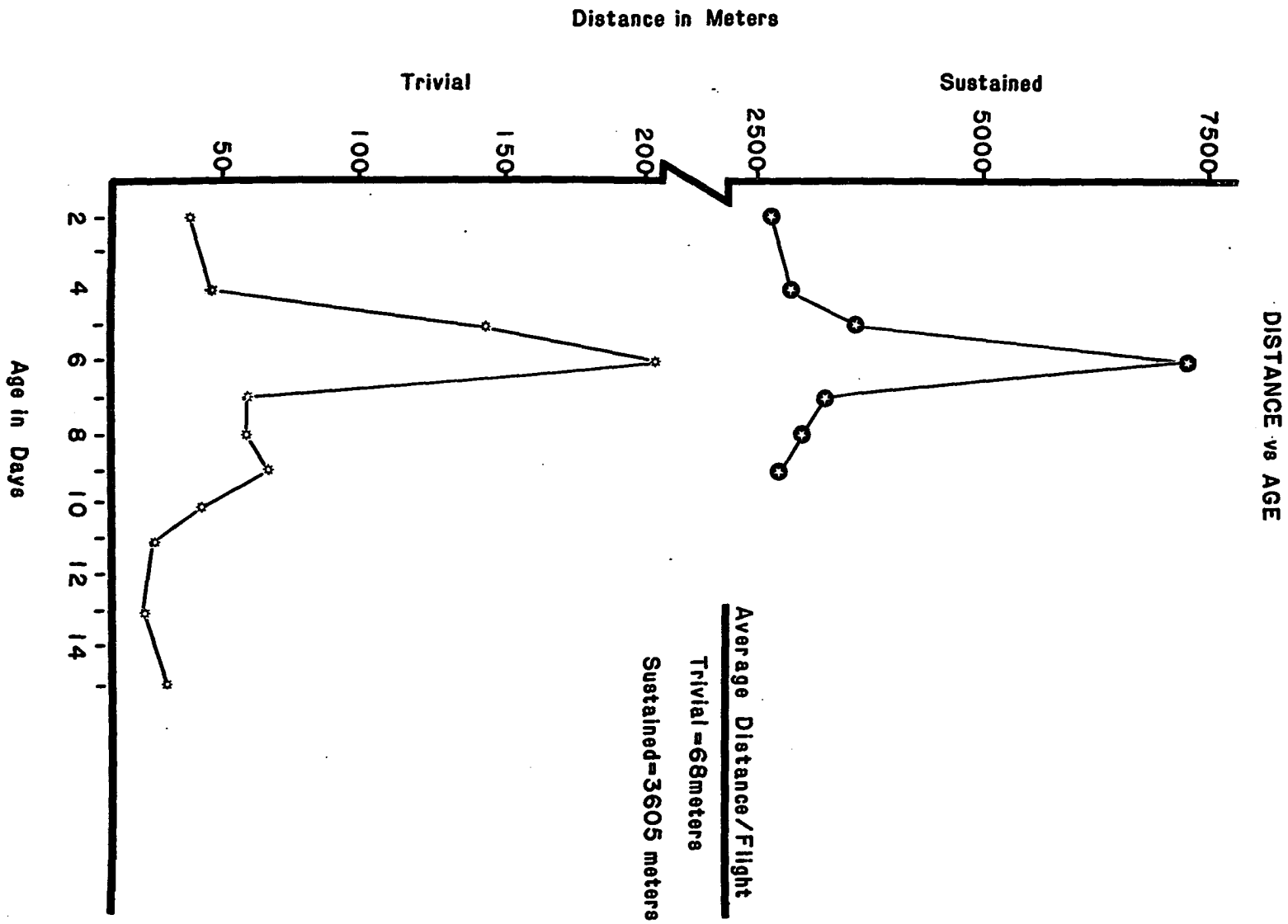
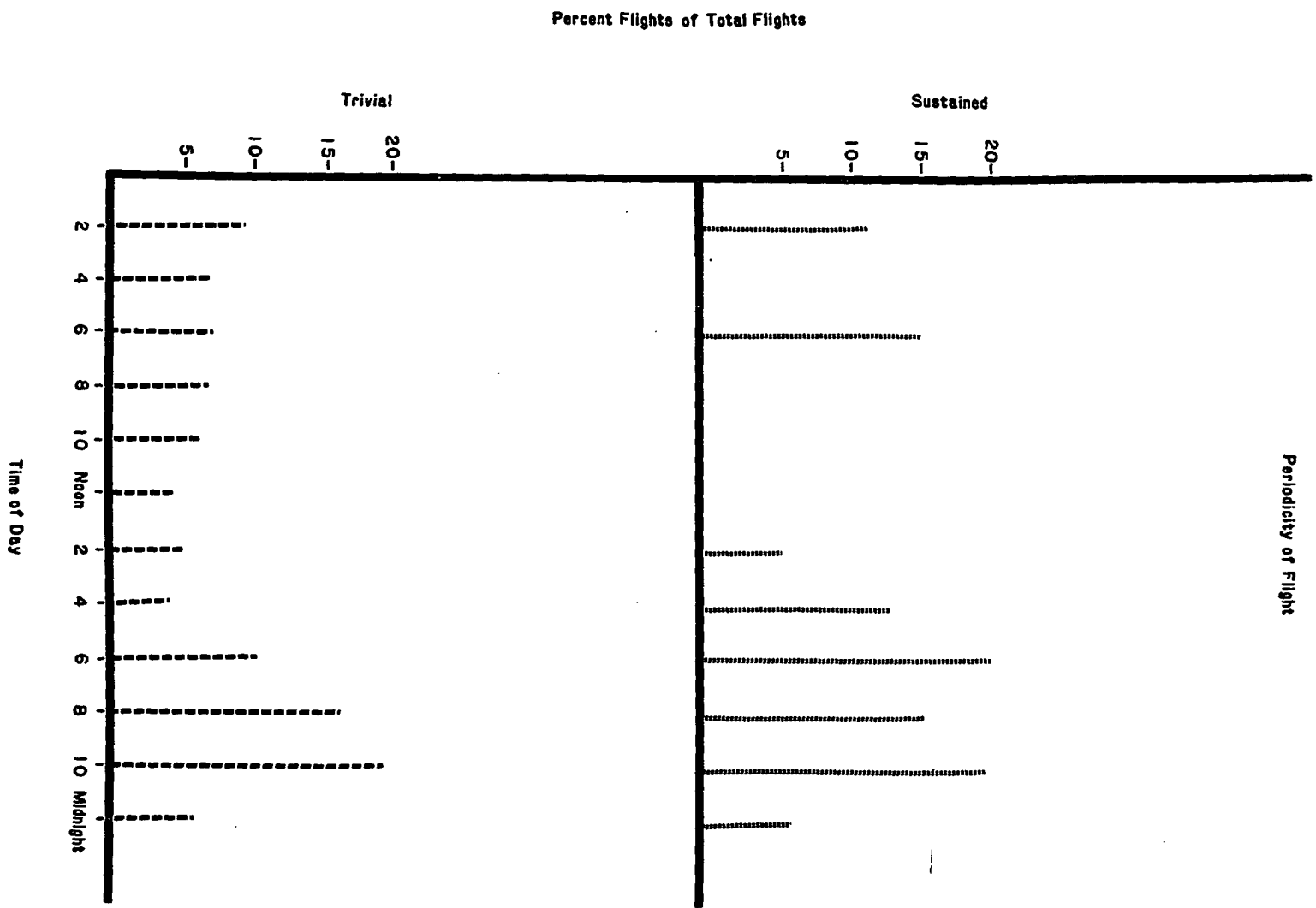


Fig. 5 Flight occurrence over time of day. Each bar represents percentage of total trivial or sustained flights that started during the hour before or after each hour marked. Note absence of migratory flight activity during the daylight hours



REGULATION OF MIGRATORY FLIGHT BY JUVENILE HORMONE MIMIC AND INHIBITOR
IN THE WESTERN CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE)

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SECTION 2. REGULATION OF MIGRATORY FLIGHT BY JUVENILE HORMONE MIMIC
AND INHIBITOR IN THE WESTERN CORN ROOTWORM (COLEOPTERA:
CHRYSOMELIDAE)

Introduction

Previous research (Coats et al. 1986) has qualitatively and quantitatively defined the flight potential in the western corn rootworm, (WCR), Diabrotica virgifera virgifera LeConte. Female beetles exhibit two types of flight: trivial and sustained. Fifteen percent of the preovipositional females made flights averaging 72 minutes in duration, which, based on accepted definitions (Dingle 1965, Tauber et al. 1984), were classified as migratory.

The catalysts of migratory flight have been the subject of several recent articles. Some of the factors which induce flight include population density, food quality, photoperiod and hormones; weather may also affect migratory movement (Rankin and Singer 1984, Tauber et al. 1984). Hormones (especially juvenile hormone (JH)) appear to be the major factors that determine the physiological changes in many insects which enable them to respond to environmental stimuli through migratory flight (Rankin 1978; Rankin and Rankin 1980a,b; Pener 1985).

Juvenile hormone produces multiple effects within species, and these effects are different from species to species. The effect produced at any particular time is determined by the level of JH in the hemolymph. The concentration in the hemolymph is controlled by the amount synthesized in the corpora allata (Englemann 1983) and the amount

degraded by JH esterases and is modulated by carrier proteins (deKort and Granger 1981, Englemann 1983). The rate of JH synthesis changes with developmental stage, and may be regulated by feedback from the ovaries and two inhibitory centers in the brain (Rankin and Stay 1984, 1985). The activity of the JH esterases is also proportional to the developmental stage and JH titre, and is under endocrine or neuroendocrine control (deKort and Granger 1981).

Different JH titres, or the different levels produced by topical applications of the JH mimic, methoprene, have been correlated with flight activity and reproduction in the large milkweed bug, Oncopeltus fasciatus and convergent lady beetle, Hippodamia convergens (Caldwell and Rankin 1972; Rankin 1974; Rankin and Rankin 1980a,b). Intermediate levels were required for migratory flight while higher levels resulted in oviposition. This relationship between flight and reproduction is supported by the correlations of low JH titres with minimal ovarian development (Lanzrein et al. 1981), and lack of flight muscle histolysis in virgin females of some species (Stay and Tobe 1977; Nair and Prabhu 1979, 1985). In mated females, ovarian maturation is associated with flight muscle histolysis and increasing JH titres (Borden and Slater 1968), and JH suppression with inhibition or regeneration of the flight muscles, providing additional evidence for the asynchronous interaction of flight with reproduction (Pener 1985, Rankin 1978, Kearney et al. 1977, Bocharova-Messner et al. 1970, Chudakova and Bocharova-Messner 1968, deKort 1969, Nair and Prabhu 1985).

Application of the JH synthesis inhibitor, precocene, has been used to confirm the importance of the juvenile hormone levels. The anti-allatotrophic activity of precocene has been demonstrated through the simultaneous inhibition of flight activity and reproductive development in the convergent lady beetle (Rankin and Rankin 1980b), and the large milkweed bug (Rankin 1980) and by temporary inhibition of flight muscle histolysis in the spruce beetle, Dendroctonus rufipennis (Sahota and Farris 1980). Therefore, the processes of flight muscle degeneration and vitellogenesis may have the same endocrine basis (Nair and Prabhu 1985).

The primary purpose of this research was to ascertain the role of JH and its effects on flight activity and reproduction in the WCR. Topical applications of juvenile hormone mimic and inhibitor were used and presumably altered levels of JH in mated females and virgin females.

Materials and Methods

Two groups of females were used for the study: mated, age-specific WCR collected daily from the field and virgin females reared individually in the laboratory. Beetles were collected daily from emergence cages during the summer of 1985 and maintained in 30.5-cm³ screened cages in a greenhouse. Windows were opened, and no artificial lighting was provided in the greenhouse, so that natural conditions of temperature and photoperiod prevailed. Lettuce, fresh corn ears, and tassels, as well as artificial diet (USDA, Brookings, South Dakota,

unpublished) were supplied daily to the beetles. These beetles were used for mated females studies.

Larvae, to rear to virgin females, were supplied by the USDA Northern Grain Insects Lab at Brookings, South Dakota. These larvae were obtained from eggs laid by field-collected females from the 1984 season. Larvae were reared to the prepupal stage in freshly germinated corn and isolated within individual 2.54 cm² cells of soil for pupation. Emerged adults were collected daily from the individual cells, sexed to obtain the females, and then maintained similarly to mated females.

The juvenile hormone mimic (JHM), methoprene, and juvenile hormone inhibitor (AJH), fluoromevalonate, were kindly supplied by the Zoecon Corporation in Palo Alto, California. These compounds were applied to both mated females and virgin females. Since preliminary testing revealed the maximal flight response occurred in females dosed with acetone solutions of 1 and 2 µg/g JHM, or 1 and 2.5 µg/g AJH, these two doses were employed. Equivalent amounts of acetone (1.0, 2.0, and 2.5 µg/g) were used for controls. These doses were applied to females two to eleven days old (day of emergence from pupa equals day 0 of adults). Each female was dosed on the venter of the abdomen at the same time on three consecutive days, the two days prior to flying, and on the day it was flown (e.g., 4-day-old females were dosed with 1 or 2 µg/g/day on days 2, 3, and 4 following emergence).

All flight testing was done on a flight mill system (Rowley et al. 1968). Each flight mill was covered by a plastic hood to prevent drafts that might confound activity and then placed in a walk-in incubator

maintained at a constant temperature of $25.6 \pm 1^{\circ}\text{C}$ and $80 \pm 5\%$ R.H. A 28-V lamp on each of the twelve flight-mill arms provided the only light. These conditions simulated twilight. For each flight, beginning and ending times and distance were recorded with a Pet Commodore 2400 microcomputer (Clarke et al. 1984).

One group of females ($n=143$) was dissected to obtain ovarian stages (Short 1970). The rest of the females ($n=468$) were mounted on the flight mill by the back of the pronotum and allowed to fly for a period of 24 h. During this 24 h period, they always remained suspended from the flight mill arms. Flights of less than one meter were not recorded by the computer. A total of 252 females, summed over all treatments, flew at least one flight of one meter or more. Each female was flown for only one 24 h period. Food, consisting of corn ears, and males, contained in 6.4 cm diameter screened cages, were placed on the floor of each hooded flight mill.

There were no statistically significant differences between the two doses used for each treatment group. Therefore, data were pooled over dose for each treatment group.

Two means were calculated for each female: one for all trivial flights and one for all sustained flights made during the 24-hour test period. These two means were used in subsequent analyses. For each age interval, ANOVAS were calculated for differences between treatments (treatments were mated females and virgin controls, JHM-dosed mated and virgins, and AJH-dosed mated and virgins). If an F test of treatments was significant at the .05 level, then least significant differences

were employed to establish which treatments were significantly different at the .05 level (Fisher's LSD, Milliken and Johnson 1984).

Results

Ovarial maturation

The stages of ovarian development for females of each treatment are presented in Table 1. Mated females require 12 to 14 days for vitellogenesis to be completed (Short 1970); the females we dissected coincided with this time period. One- to three-day-old beetles had undifferentiated cells in the anterior part of the germarium, with no oöcytes in the vitellarium. In four- to six-day-old beetles, young oöcytes had proceeded into the vitellarium. At seven days of age, yolk deposition had begun. At eleven days, yolk deposition was well underway, but no chorion was present. Chorionated eggs were visible in a freeze-fractured female abdomen twenty days old. No egg development past the undifferentiated stage was noted for virgin controls.

Application of JHM increased the rate of ovariole development. Treated females possessed numbers of oöcytes characteristic of controls four to ten days older. The largest increase in developmental stages of the ovaries of treated females over controls was noted for ages seven through eleven. JHM treated virgins possessed ovaries equivalent to the mated controls; almost identical development through day eleven was noted.

The effect of the use of inhibitor on virgins revealed no noticeable difference between such treatment and the control virgins.

Only undifferentiated cells were noted in the germarium. Mated females treated with inhibitor had a slower rate of egg development than mated controls. Oöcytes of AJH treated six-day-olds were just appearing in the vitellarium, although after ten days of age, there was less difference from the control than at the younger ages.

Short flight activity

Controls Flight times and distances are presented in Table 2. Average flight times were 4.8 min for mated females and 6.9 min for virgin female controls (Fig. 1a). Average distances per flight were 2.2 m for mated females and 3.8 m for virgin controls (Fig. 1b). Thirty percent of the virgin females flew compared to 56% of the mated females (Table 3). Virgins only flew from days three through five. Mated females flew on all days tested. While virgins flew for statistically significant longer times and greater distances on a per flight basis, number of flights and total distances flown on a per female basis were greater for mated females than for virgin females: 28.6 flights and 57.0 m (mated) and 2.2 flights and 22.2 m (virgins). Flight activity peaked on day three for virgin controls, while trivial flights were longest for mated controls on day six. Significant differences in flight times of the two controls existed on days two to four, and six to eleven.

JHM-treated females Application of JHM removed most significant differences between mated and virgin female flight activity. Average flight times and distances per flight were 5.8 min, 63.4 m for mated,

and 5.0 min, 66.6 m for virgin (Table 2). Means of total flight times and distances per female increased dramatically over controls: 155 min, 1696.2 m for mated and 131.8 min, 1755.8 m for virgins. The mean number of flights/female remained constant (26.8 mated and 26.4 virgin) but the percentage of females flying rose from 30 to 50% over controls (Table 3). The JHM-treated virgins flew from days two through ten, slightly outpacing the mated females in total days flying (Fig. 2a) and distances flown over days (Fig. 2b). Peak flight activity for both mated and virgin females was poorly defined, and displaced over a four to five day interval. A significant difference between JHM-treated female groups was noted for days two, nine, and ten.

AJH-treated females Fluoromevalonate completely inhibited all flight activity by virgins (Fig. 3), producing significant differences between mated and virgin females for days four to eleven. The average flight times and distances for mated females treated with AJH were 7.8 min and 79.4 m per flight (Table 2). On a per female basis, a mean of 297.8 min were spent flying an average total distance of 3031.1 m. Each female flew an average of 38.2 flights, and 63.3% of all tested females flew (Table 3). Peak flight activity occurred on days five and six, but a considerable amount of flying continued through day eleven, the last day tested.

Combined analysis for virgin females Fig. 4 illustrates the effects on JHM and AJH compared to the control. Significant differences occurred between the AJH-treated females and the control for ages three through five. There were significant differences between the JHM and

AJH treatments over all days, except day eleven. The JHM-treated females were significantly different from the control virgins at days three and six through ten.

Combined analyses for mated females Flight-activity of AJH-treated mated females is a remarkable facsimile of the mated control (Fig 1a), except at a higher level and delayed until day three (Fig. 5). The application of inhibitor appears to increase flight, while the JHM-treatment causes a general decrease in flight compared to the control, possibly due to the earlier reproductive development. Significant differences were found at days three and six through eleven for AJH and control, at days two, three, and six through eleven for AJH and JHM and at days two, three and seven through eleven for JHM and control.

Long flight activity

Controls No long flight activity occurred in any virgin females (Fig. 6). The average flight time and distance for mated females was 47.3 min and 1901 m. There was an average of three flights/female, with 28.9% of the females tested performing long flights (Table 3). Peak flight activity occurred on day five, with no long flights occurring before day three or after seven. Significant differences between mated and virgin females occurred for days 2, 5, and seven.

JHM-treated females The use of methoprene on virgin and mated females produced a significant increase in flight activity (Fig. 7a,b). Mated females averaged 71.0 min and 1472.4 m per flight. Virgins flew a mean of 96.8 min and 3470.6 m per flight (Table 2). Means of 3.4

flights per mated female, and 1.9 flights per virgin were noted. Forty percent of the mated females, and 52% of the virgins flew sustained flights (Table 3). Peak activity occurred on day four for mated females compared to day seven for virgins (Fig. 7a). Virgins flew on days two through nine, while long flights by mated females ended after day five. Significant differences between mated females and virgins occurred on day seven. The largest difference in flight means between any two groups occurred with the JHM treatment: 15 km for virgins compared to 2 km for mated females on day seven.

AJH-treated females The inhibitor again totally negated flight activity of virgins. It produced an increase in flight activity in mated females over controls (Fig. 8). Mean flight time and distance were 59.9 min and 914.2 m. Over twice as many AJH-treated females flew long flights as controls (Table 3). Each female averaged 2.5 flights. Flight activity was inhibited in mated females before day four, but lasted through the last day (eleven) tested. Peak activity occurred on day seven, with a mean flight distance of 1751 m (Table 2), but substantial flight times and distances were present through day ten. Significant differences occurred between AJH mated females and control mated females from days six through eleven.

Combined analysis for virgin females The effect of JHM on virgins was dramatic (Fig. 9). While there were no long flights by the control or AJH-treated females, there was a phenomenal response to the application of JHM. Long flights by two- through seven-day-old females occurred with JHM treatment. Virgin flight activity was almost double

that of JHM-treated, mated females. Statistically significant differences occurred on days five through seven.

Combined analysis for mated females The obvious trend shown by Fig. 10 is the effect of treatment on the peak flight activity relative to day. Application of JHM produces the maximal sustained flight activity in younger beetles. The inhibitor delays activity until the beetles are older. Peak flight activity occurs in JHM-treated beetles that are four days old, in control beetles peak activity occurs on day five, and on day seven with AJH-treated beetles. The earlier activity peak in JHM-treated females is followed by cessation of long flights after day five, while control females make long flights through day seven. AJH treatment inhibits long flight activity in two- and three-day-old beetles, but extends sustained flight activity through day eleven. This is four days later than peak activity in controls. The flight times on days two and seven through eleven proved significantly different for the JHM and AJH treatments. Response of beetles to AJH treatment was significantly different from the control on days seven through eleven.

Another trend is the increased flight activity observed in both JHM and AJH treated females compared to control beetles. More treated females flew (Table 3), and the distances and times they flew were greater than that of the controls (Table 2).

Discussion and Conclusions

Methoprene (JHM) effects

The main treatment effects of JHM included longer flight times and distances and a greater number of flights per female. A much larger percentage of treated females flew both short and long flights compared to controls. Application of artificial JH obviously induces a significantly greater flight response compared to controls. More rapid development of the ovaries also occurred with JHM treatment. Other researchers also found this to occur in Hippodamia convergens (Rankin and Rankin 1980b).

A displacement of flight behavior over age for beginning, ending, and peak activity was noted for both trivial and sustained flights. For mated females treated with JHM, peak flight activity was induced two days sooner and ended several days earlier than untreated mated females. In previous research (Coats et al., Section 1, herein), mated females still flew short distances on day fifteen, the last age tested. Long flight activity of mated females continued to day nine in the controls. Long flights were not attempted by JHM-treated mated females after day five. Ovarial development of JHM-treated mated females proceeded very rapidly compared to the untreated mated control. At the age of three days post emergence, mated females treated with JHM were one day advanced, but by seven days they were four days beyond their untreated, mated counterparts. Change in the rate of ovarian development coincides with the abbreviated flight pattern of the JHM-mated individuals.

Virgin females were influenced significantly by JHM treatment. Long flights of virgins were produced by JHM treatment where there were none in the controls. Peak activity occurred at day seven, rather than at day five in the untreated, mated females. This delay may be caused by inherent physiological differences in mated females vs virgin females. Mating may produce multiple alterations in the physiology and biochemistry of this insect, while the experiments here only change the JH level. For example, mating stimulates ovarian development in the WCR (Hill 1975). Although ovarian development in JHM-treated virgins seems to correlate with untreated mated females, feedback from vitellogenic ovaries (Rankin and Stay 1984) may be delayed when a beetle comes in contact with a large artificial supplement of JH compared to the gradual increase that normally occurs.

Short-flight activity does occur in virgin control beetles. It endures for just the first five days compared to the minimum of 15 days for mated female controls. The application of JHM seems to delay flights until older ages, and produces a large spread of activity from ages three to eleven.

Fluoromevalonate (AJH) effects

Although AJH completely inhibited all flight activity in virgins, it produced the greatest percentage of flying individuals among all treatments in the mated females. Longer times and distances and a greater number of flights per mated female were recorded for AJH-treated individuals. The peak number of short flights per female was 38.2.

Compared to controls, mean times and distances were 50% greater for short flights; flight times were 20% longer and distances 30% greater for long flights.

AJH consistently affected sustained flights in older females compared to the controls. The peak of activity (day seven) was two days later than for controls. Thus the onset of migratory flight is delayed two days, but it continues two days longer than in controls. This time lag relates well to ovarian development which is also two days later than in controls. However, the AJH effect on ovaries is not as pronounced in females nine- through eleven-days-old (but neither is long flight activity).

AJH and JHM treatment increased short flight activity. Unlike JHM treatment, AJH treatments caused minimal age-displacement in flight activity. A postponement of flight activity occurred for one day in treated females, but the flight times and distances surpass the control on the next day, and peak at the same time. Instead of inhibiting short flight activity, fluoromevalonate actually stimulated it. Since AJH postpones sustained flight activity until later ages, it seems plausible that it releases short-flight activity from the constraints imposed by mechanisms affecting long flights. Since long flights require critical concentrations of JH and large energy stores (Rankin and Singer 1984), restriction of long flights may make energy resources more available for short flights. There may be many complex interactions occurring with AJH treatments that are explained by these data. Further studies,

including assessment of JH concentrations in the hemolymph of the WCR, will be necessary to determine these.

Interpretation of JH effects on flight behavior in adult females

The age displacement of peak flight activity affected by JHM and AJH suggests there is a critical level of JH associated with migratory flight in this species. Long flight activity in virgins and very young, mated females is absent, and is artificially produced by JHM application. The extra dose of JH applied through JHM treatment to mated females (in addition to internal production levels) causes an early cessation of sustained flight and premature ovarian development. The inhibition of JH synthesis extends the migratory period while delaying ovarian development. The negative correlation between vitellogenesis and sustained flight reported in the literature (Nair and Prabhu 1985) is confirmed. Topical application of a JH mimic and JH inhibitor to female WCR is correlated with ovarian development and flight behavior.

Female WCR adults maintain short flight capabilities throughout their lifetime of up to two months (personal observation) but can only migrate from two to ten days post emergence. The interpretation of short flight data produced when JH levels were artificially altered is difficult. Treatment with JHM or AJH affects an "all or none" migratory response delineated by the 30 min parameter. Short flight activity occurs in all treatment groups except one (AJH treated virgin females). The degree of response affected by treatments must be evaluated for

short flight activity. Defining characteristics associated with different degrees of response is more complicated than ascertaining the number of 30-min flights for the migratory response.

Summarizing short flight data, JHM and AJH cause increased flight activity in mated females and virgins over controls. For controls: (1) virgins fly short flights (but not long flights), (2) flight activity only occurs from days three to five in virgin females, (3) activity peaks on day five in mated females, but occurs on all days tested (except day two). For JHM treated: (1) virgin flight activity occurs from day two to ten, but peaks on day seven in virgin JHM-treated females, (2) Activity occurs on days two to seven, peaking on day four in mated females. For AJH Treated: (1) virgins do not fly, (2) mated females start flying one day later in life, but perform the same as the mated female control over age, except with an increased level of activity.

These results indicate that JH level is an important factor in short flight activity. To interpret the significance of JH level over the span of the female adult's lifetime, factors vital to the insect must be considered. Mating, food, and oviposition sites are candidate factors. Short flight activity in virgins immediately after emergence may correspond to locating a mate. A beetle not mated prior to a long flight would have a much smaller chance of encountering males.

Mated females seek food and oviposition sites. Thus, untreated mated females should have a peak flight activity on day five; the time when it is imperative that she find adequate energy for egg maturation.

It is also reasonable to expect short flight activity to continue in mated females since they continue to require food and continue to deposit clutches of eggs in different locations over five day intervals (Hill 1975).

The cessation of flight activity by virgins at day five may be related to ovarian maturation. The ovary serves as a regulator of JH synthesis, increasing the titre (Rankin and Stay 1984). If mating does not occur, the ovaries fail to develop (Table 1) (Hill 1975). This could be due to low JH titre in the hemolymph (as found for the cockroach, Nauphoeta cinerea (Lanzrein et al. 1981)). Subsequent feedback to the corpora allata may not occur and the catalyst for continued short flight activity (to find oviposition sites) is lost. Alternatively, if increased JH synthesis is associated with oöcytes of vitellogenic ovaries, and short flight activity is necessary to locate oviposition sites, varying JH levels may be the mechanism that stimulates short flight activity in mated females. If oöcytes of mated females are matured at a faster rate with JHM-treatment, the need to locate oviposition sites would be accelerated, and a high level of short flight activity would be stimulated. This would explain why JHM-treatment produces a higher level of short-flight activity than in the controls. Flight activity ceases when too high a level of JHM is applied, possibly due to flight muscle histolysis (as found in Dysdercus lingulatus (Nair and Prabhu 1985)). When a JH inhibitor is applied, it maintained a lower level of JH which stimulated short flight activity over the entire range of days tested (through day eleven).

The level of JH in the hemolymph is age-regulated by JH esterases, ovarian feedback, and JH synthesis in the corpora allata, and is moderated by carrier proteins. Artificially supplementing JH levels in the hemolymph or reducing JH synthesis may result in additional regulation by these factors. Although the JH mimic and inhibitor are nontoxic and simulate the effects produced by the natural JH (Henrick 1982), all the effects of JH, JHM, and AJH are not fully understood. Clearly, more work is required to elucidate fully the role of juvenile hormone in the regulation of flight in this insect.

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Table 1. Ovarial development stages of all ♀♀^a according to treatment^b. Percent of ♀♀ (n=143) in a given stage is indicated in parentheses

Age of ♀♀ (days)	Treatments					
	Mated (Control)	Mated (JHM)	Mated (AJH)	Virgin (Control)	Virgin (JHM)	Virgin (AJH)
2	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
3	1 (100)	1 (70) 2 (30)	1 (100)	1 (100)	1 (100)	1 (100)
4	1 (70) 2 (30)	2 (80) 3 (20)	1 (100)	1 (100)	1 (70) 2 (30)	1 (100)
5	2 (80) 3 (20)	3 (30) 4 (70)	1 (100)	1 (100)	2 (80) 3 (20)	1 (100)
6	2 (60) 3 (40)	3 (70) 4 (30)	1 (60) 2 (40)	1 (100)	2 (60) 3 (40)	1 (100)
7	2 (40) 3 (60)	3 (90) 4 (10)	2 (90) 3 (10)	1 (100)	2 (40) 3 (60)	1 (100)
9	2 (20) 3 (80)	4 (40) 5 (60)	2 (70) 3 (30)	1 (100)	2 (20) 3 (80)	1 (100)
10	3 (90) 4 (10)	4 (10) 5 (90)	2 (30) 3 (70)	1 (100)	3 (100)	1 (100)
11	3 (80) 4 (20)	5 (100)	3 (90) 4 (10)	1 (100)	3 (80) 4 (20)	1 (100)

Stage 1: Early development, no oocytes in vitellarium						
Stage 2: <5 oocytes in vitellarium						
Stage 3: >5 oocytes in vitellarium; yolk deposition in progress in these oocytes						
Stage 4: Yolk deposition nearly complete in oocytes, but no chorionated eggs present						
Stage 5: Fully developed eggs with chorion but no eggs in calyx						

^aModified from Short 1970: These stages of 1, 2, 3, 4, and 5 correspond to his stages of 1, 1.5, 2-2.5, 3, and 3.5, respectively.

^bTreatments = Control: mated and virgin ♀♀ dosed with acetone;
JHM: mated and virgin ♀♀ dosed with methoprene;
AJH: mated and virgin ♀♀ dosed with fluoromevalonate.

Table 2. Mean flight times, distances, and number of flights for all treatments and ages^a

Treatment											
Age	Mated Control ^b		Virgin Control		Mated JHMC		Virgin JHM		Mated AJH ^d		
	Short	Long	Short	Long	Short	Long	Short	Long	Short	Long	
2	X ^e	0	0	0	0	5.0	57.0	7.0	39.0	0	0
	Y ^f	0	0	0	0	45.0	1284.3	81.0	358.0	0	0
	Z ^g	0	0	0	0	28.0	7.0	12.0	4.1	0	0
3	X	2.3	46.3	8.6	0	6.8	68.0	2.6	46.3	0	0
	Y	52.5	170.0	30.3	0	54.7	1497.0	48.2	1480.0	0	0
	Z	1.5	0.5	3.6	0	38.7	2.2	46.0	1.3	0	0
4	X	3.5	42.4	5.2	0	7.4	112.0	7.4	49.9	6.5	0
	Y	35.0	305.0	15.0	0	78.0	2941.1	83.4	980.6	70.0	0
	Z	24.4	0.4	1.3	0	51.7	1.5	49.5	0.8	1.0	0
5	X	6.5	67.9	6.8	0	5.8	47.3	6.8	45.0	8.0	52.9
	Y	78.8	1560.1	21.2	0	32.4	167.0	72.8	1022.5	121.2	298.0
	Z	65.4	4.3	2.0	0	17.4	4.2	99.0	1.2	26.0	1.2
6	X	7.6	48.1	0	0	4.6	0	6.2	125.4	10.5	39.3
	Y	76.1	964.3	0	0	102.8	0	83.1	1342.5	86.6	628.0
	Z	48.2	3.6	0	0	1.2	0	2.4	1.2	26.0	1.7
7	X	5.3	31.8	0	0	5.5	0	7.0	275.0	7.5	91.4
	Y	20.0	170.0	0	0	100.0	0	102.0	15640.0	86.2	1751.4
	Z	17.0	3.0	0	0	1.0	0	2.6	1.5	39.3	5.4
9	X	4.5	0	0	0	0	0	2.0	0	7.3	75.6
	Y	40.0	0	0	0	0	0	35.0	0	47.0	1567.1
	Z	8.5	0	0	0	0	0	0.5	0	42.4	2.4

10	X	3.5	0	0	0	0	0	1.0	0	6.4	66.7
	Y	24.4	0	0	0	0	0	27.0	0	50.7	1642.7
	Z	2.6	0	0	0	0	0	0.3	0	46.0	1.6
11	X	5.6	0	0	0	0	0	0	0	8.6	33.7
	Y	58.1	0	0	0	0	0	0	0	94.0	512.5
	Z	12.3	0	0	0	0	0	0	0	13.2	1.0

^aNo flights were made by virgin females treated with AJH, so this treatment is excluded.

^bControls treated with acetone.

^cJHM = juvenile hormone mimic, methoprene.

^dAJH = juvenile hormone inhibitor, fluoromevalonate.

^eX = mean flight time (min) per flight/♀.

^fY = mean distance (meters) per flight/♀.

^gZ = mean number of flights/♀.

Table 3. Tabulation of number of ♀♀ who flew short flights (X), and long flights (Y), and total number of ♀♀ tested (Z)

Treatment		Ages									X	Totals ^a	
		2	3	4	5	6	7	9	10	11		Y	Z
Mated Control ^b	X	0	6	7	12	7	7	4	6	5	<u>54</u>	[55.7%]	
	Y	0	2	4	9	6	7	0	0	0		<u>28</u>	[28.9%]
	Z	16	8	11	12	16	7	9	8	10			<u>97</u>
Virgin Control	X	0	7	10	7	0	0	0	0	0	<u>24</u>	[30.3%]	
	Y	0	0	0	0	0	0	0	0	0		<u>0</u>	[0.0%]
	Z	6	9	12	10	7	14	8	7	6			<u>79</u>
Mated JHM ^c	X	15	19	11	12	9	7	0	0	0	<u>73</u>	[78.5%]	
	Y	6	12	10	9	0	0	0	0	0		<u>37</u>	[40.0%]
	Z	15	19	12	13	11	8	6	5	4			<u>93</u>
Virgin JHM	X	4	6	6	5	8	7	5	3	0	<u>44</u>	[84.6%]	
	Y	4	3	6	5	5	4	0	0	0		<u>27</u>	[51.9%]
	Z	5	7	6	5	9	7	6	3	4			<u>52</u>
Mated AJH ^d	X	0	0	1	4	3	12	14	19	4	<u>57</u>	[63.3%]	
	Y	0	0	0	4	3	11	14	17	4		<u>53</u>	[58.9%]
	Z	7	10	5	8	7	12	14	20	7			<u>90</u>
Virgin AJH	X	0	0	0	0	0	0	0	0	0	<u>0</u>	[0.0%]	
	Y	0	0	0	0	0	0	0	0	0		<u>0</u>	[0.0%]
	Z	6	8	9	7	6	5	5	4	7			<u>57</u>
Total number short flights											<u>252</u>	[54%]	
Total number long flights												<u>145</u>	[31%]
Total number ♀♀ tested													<u>468</u>

^aPercentages of ♀♀ who flew flights of one meter or more.

^bControls dosed with acetone.

^cJHM = Juvenile hormone mimic, methoprene.

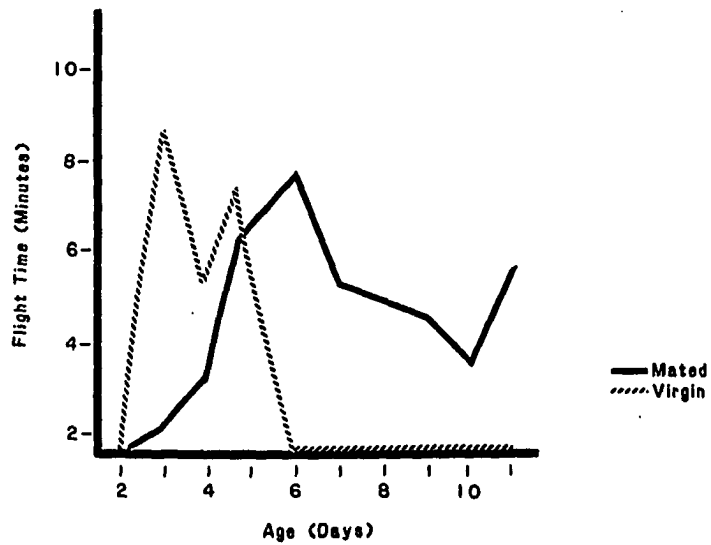
^dAJH = Juvenile hormone inhibitor, fluoromevalonate.

Fig. 1. Short flight activity of mated and virgin controls.

Fifty-four mated ♀♀ flew 1545 flights, 24 virgin ♀♀ flew 53 flights

- a. Mean flight time/flight/♀ are plotted vs age. Note the longer flight times of virgins at early ages, but short distances flown indicating much slower flight speeds compared to mated**
- b. Mean total distance/flight/♀ are plotted vs age**

SHORT FLIGHT ACTIVITY
FLIGHT TIMES of CONTROLS



SHORT FLIGHT ACTIVITY
FLIGHT DISTANCES of CONTROLS

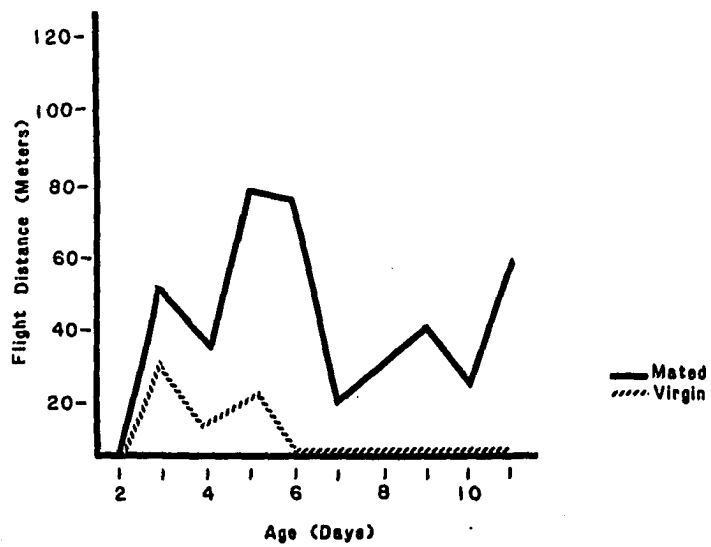
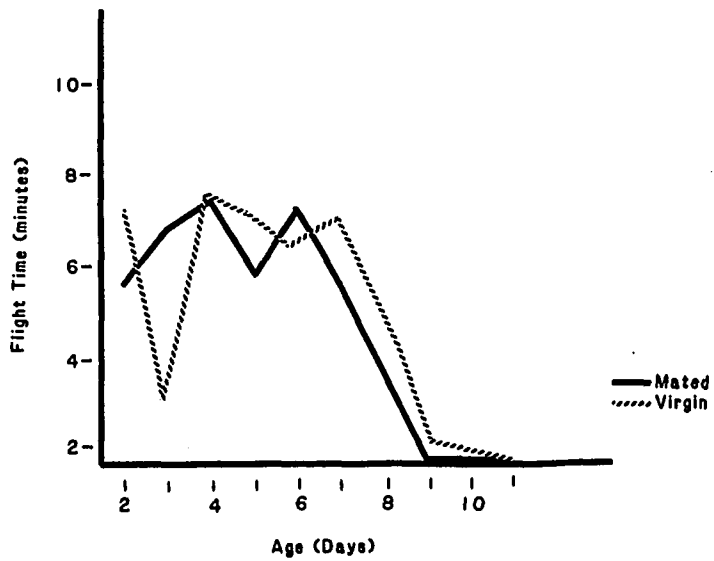


Fig. 2. Short flight activity of mated and virgin ♀♀ treated with JHM. Seventy-three mated ♀♀ flew 1957 flights, 44 virgins flew 1162 flights

- a. Mean flight time/flight/♀ are plotted vs age
- b. Mean flight distance/flight/♀ are plotted vs age

SHORT FLIGHT ACTIVITY
FLIGHT TIMES of JHM-TREATED FEMALES



SHORT FLIGHT ACTIVITY
FLIGHT DISTANCES of JHM-TREATED FEMALES

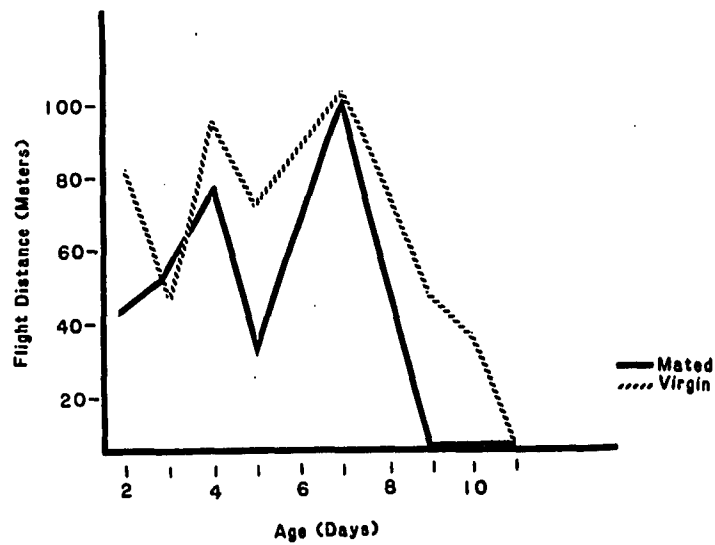


Fig. 3. Short flight activity of mated and virgin ♀♀ treated with AJH.

Fifty-seven mated ♀♀ flew 2178 flights, no virgin flew any flights.

Mean flight time and distance/flight/♀ are plotted vs age. Note the slight increase in time required/flight indicating slower speeds at older ages

SHORT FLIGHT ACTIVITY
FLIGHT TIMES and DISTANCES of AJH-TREATED FEMALES

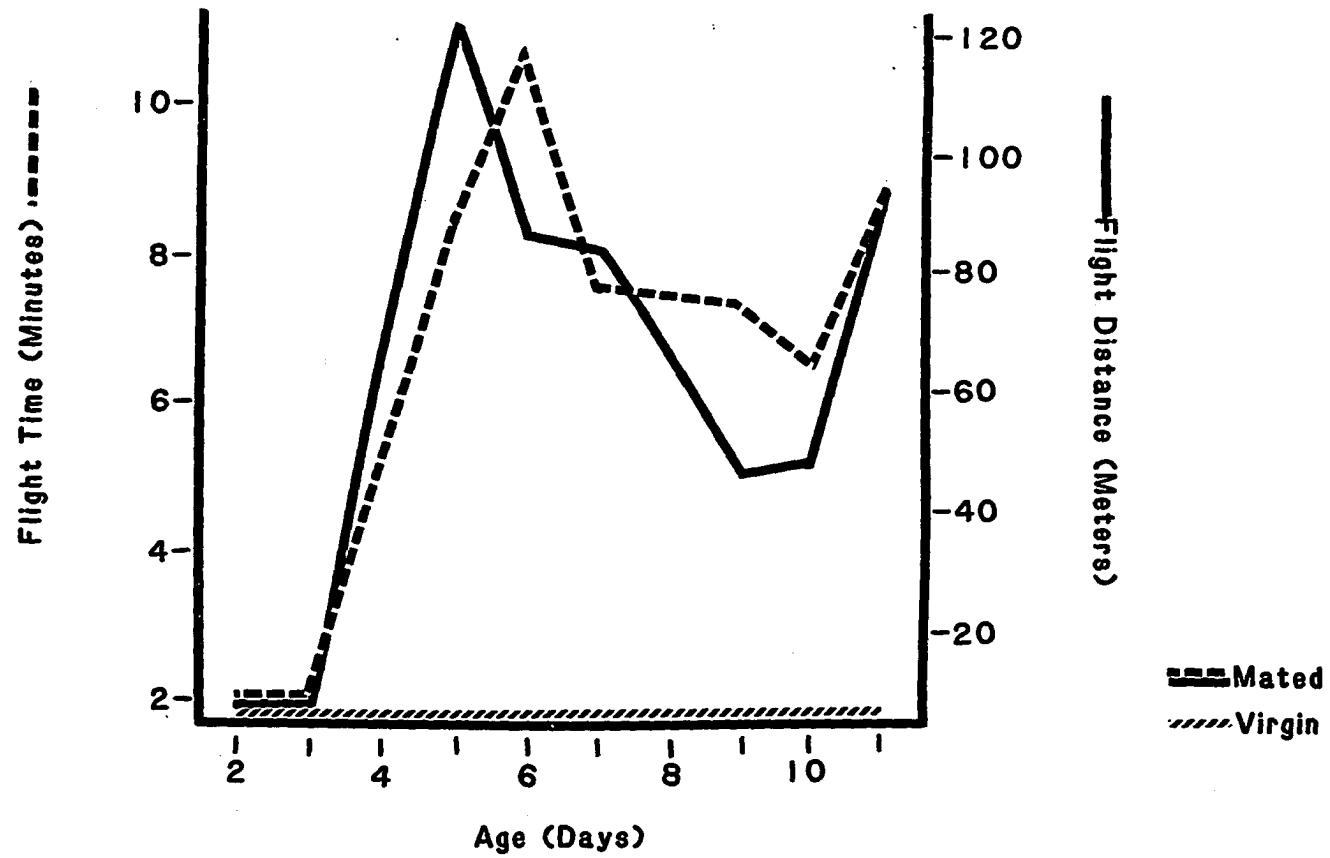


Fig. 4. Combined analysis of short flight activity for all virgins used in control, JHM, and AJH treatments. Note the prolonged flight activity for JHM-treated ♀♀ when compared to control, and no flights by AJH treated ♀♀

SHORT FLIGHT ACTIVITY
FLIGHT TIMES of VIRGIN FEMALES

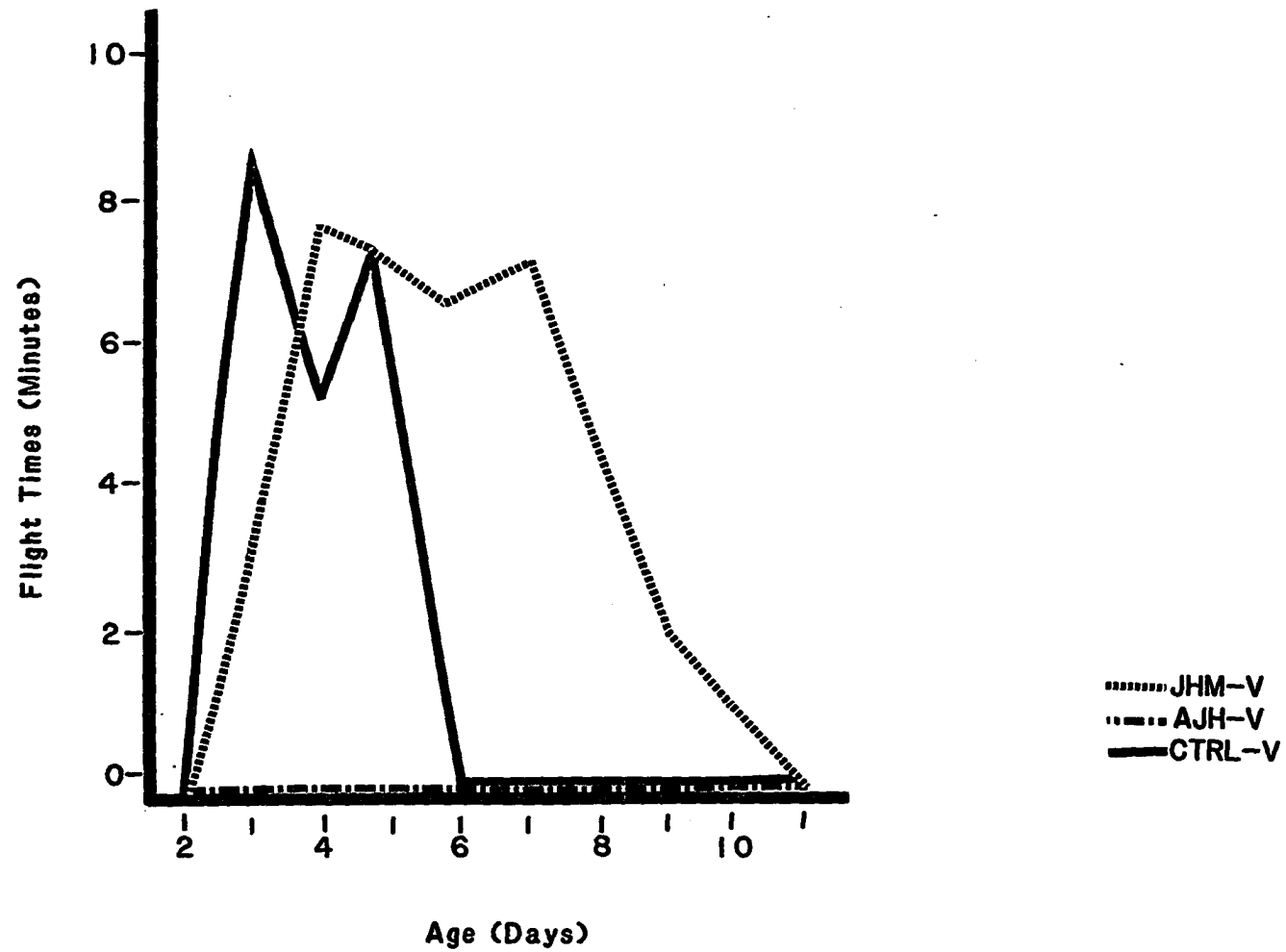


Fig. 5. Combined analysis of short flight activity for all mated ♀♀ used in control, JHM, and AJH treatments. Note the earlier flight activity of the JHM-treated ♀♀ and the increased amplitude of flight time for AJH-treated ♀♀ compared to the control

SHORT FLIGHT ACTIVITY

FLIGHT TIMES of MATED FEMALES

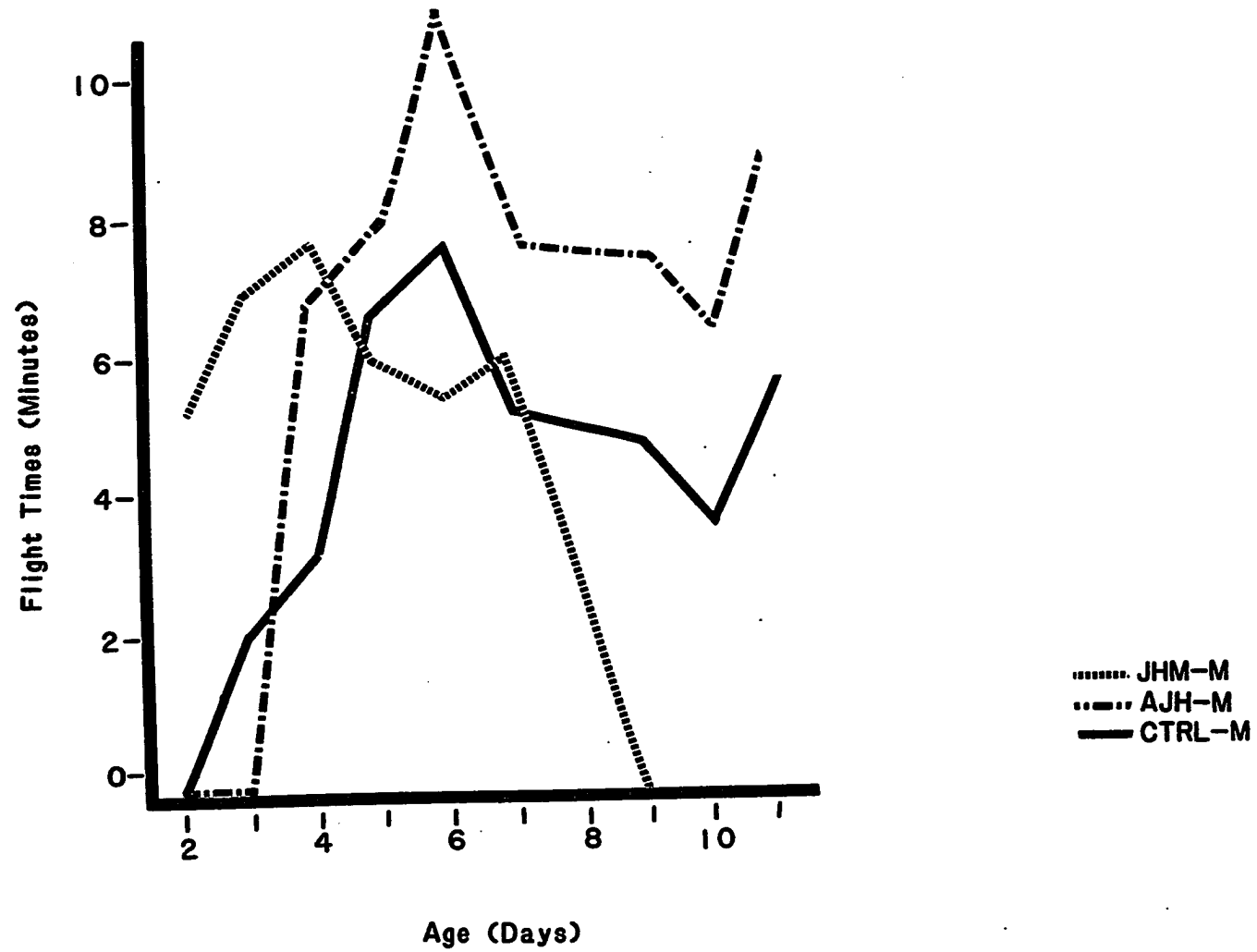


Fig. 6. Long flight activity of virgin and mated controls. Mean time and distance/flight/♀ are plotted vs age. Twenty-eight mated ♀♀ flew 117 flights, no virgin ♀♀ flew any flights

LONG FLIGHT ACTIVITY
FLIGHT TIMES and DISTANCES of CONTROLS

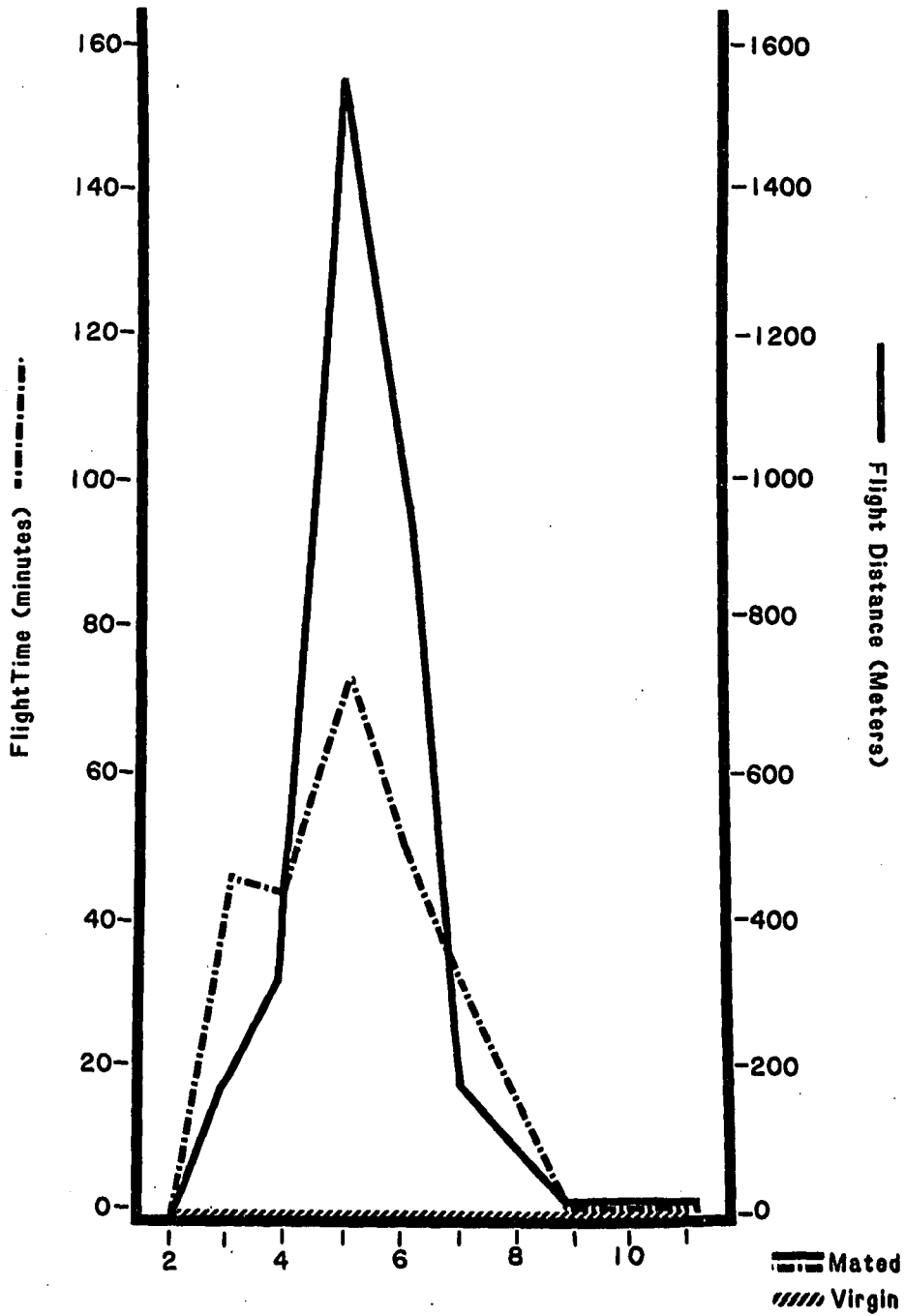
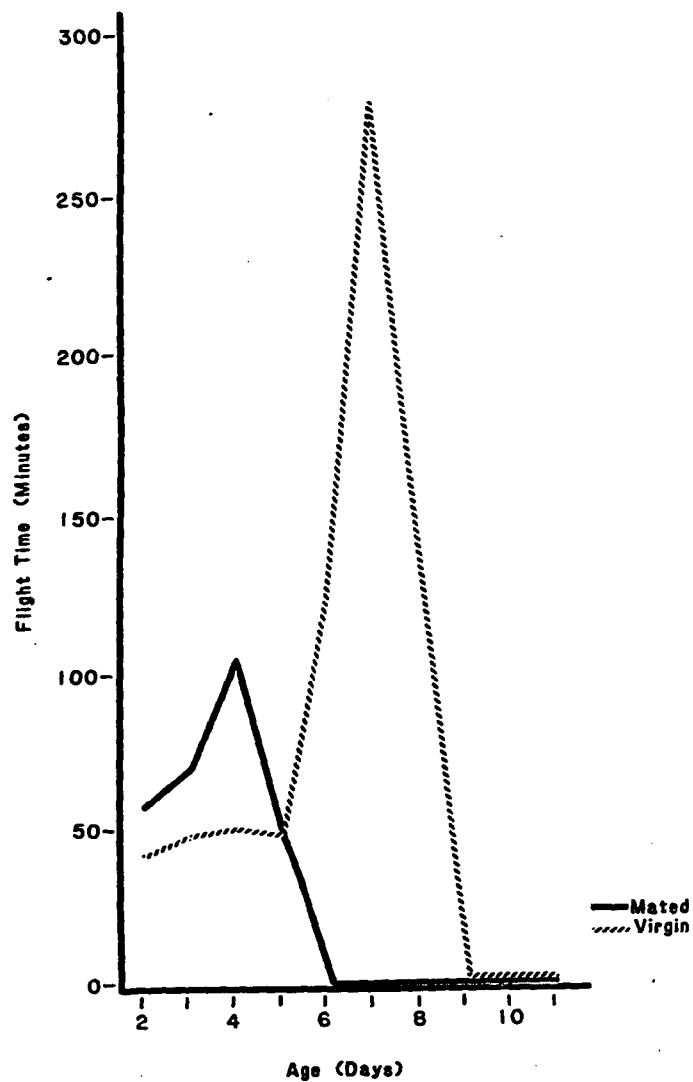


Fig. 7. Long flight activity of virgin and mated ♀♀ treated with JHM.

Thirty-seven mated ♀♀ flew 126 flights, 27 virgin ♀♀ flew 51 flights

- a. Mean flight time/flight/♀ are plotted vs age. Note the ages at which the peak flight times occurred for each treatment group
- b. Mean flight distance/flight/♀ are plotted vs age. Note cessation of flight activity for JHM-treated mated ♀♀ after age five

LONG FLIGHT ACTIVITY
FLIGHT TIMES of JHM-TREATED FEMALES



LONG FLIGHT ACTIVITY
FLIGHT DISTANCES of JHM-TREATED FEMALES

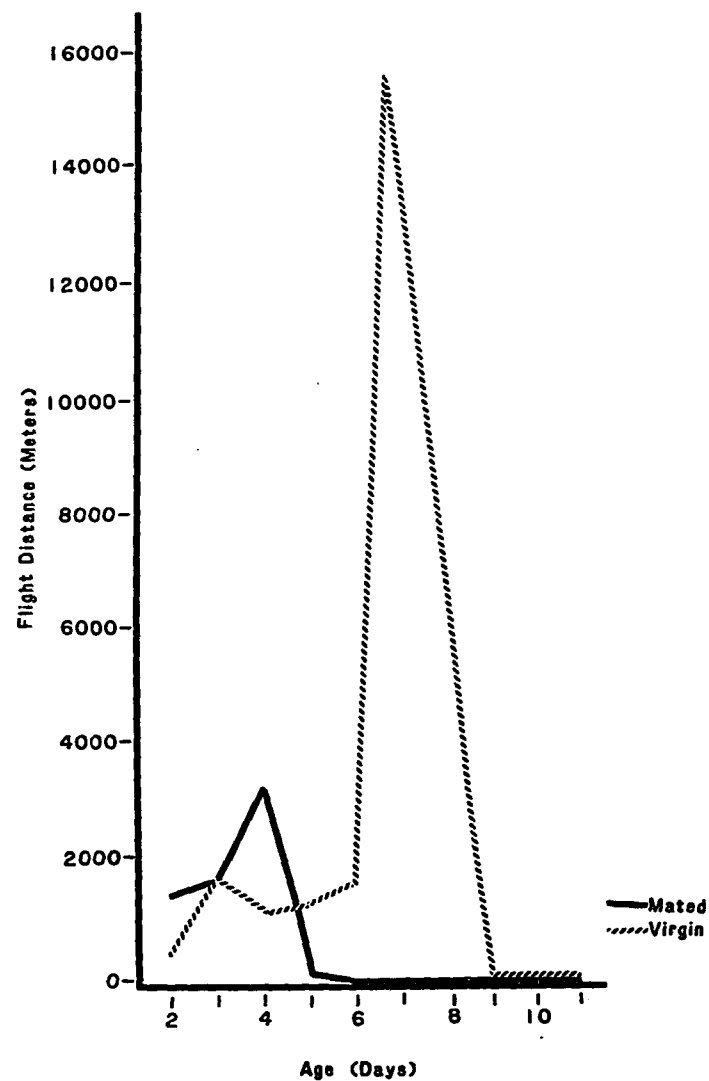


Fig. 8. Long flight activity of AJH-treated ♀♀. Mean flight time and distance/flight/♀ are plotted vs age. No virgin flights occurred, 53 mated ♀♀ flew 133 flights. Note the broad peak of flight activity from ages 6-10

LONG FLIGHT ACTIVITY
FLIGHT TIMES and DISTANCES of AJH-TREATED FEMALES

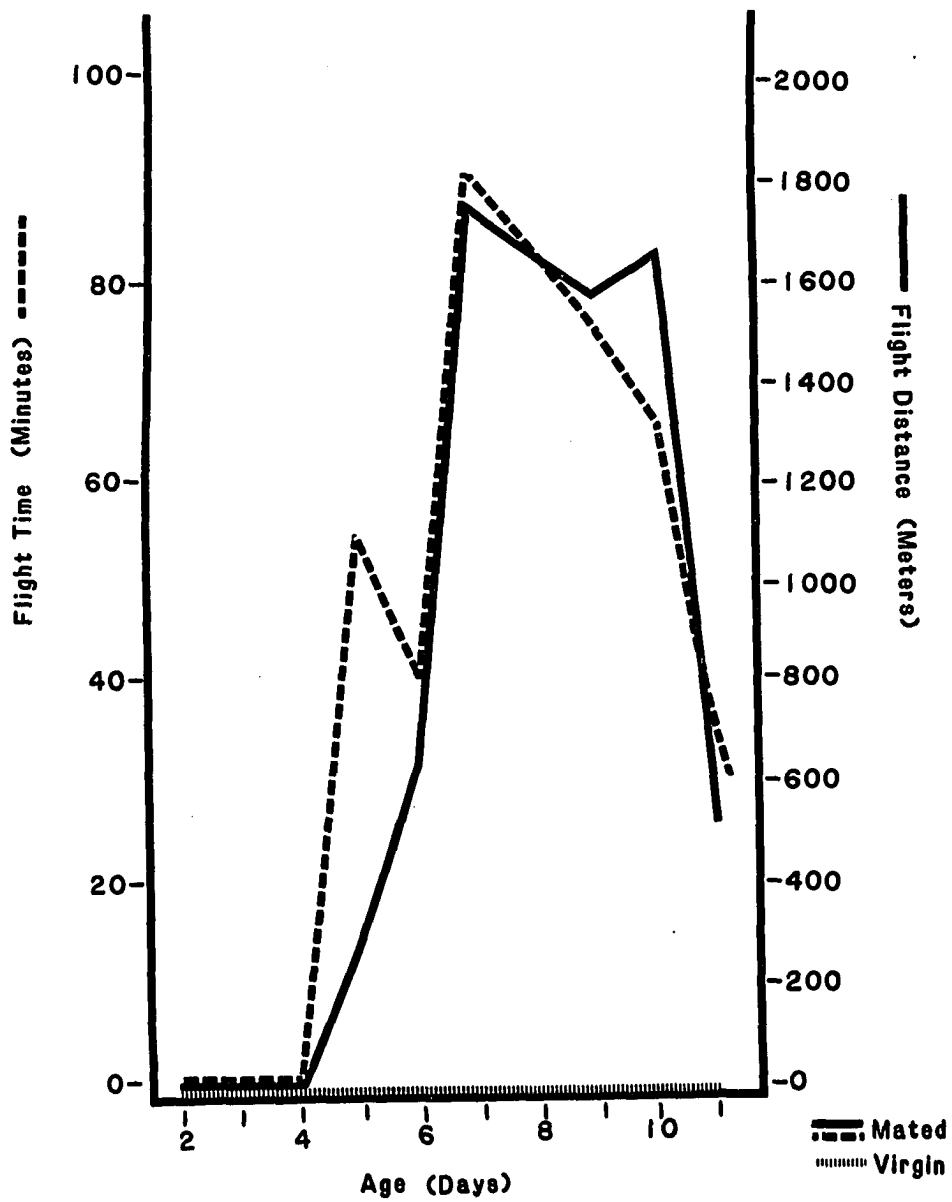


Fig. 9. Combined analysis of long flight activity for all ♀♀
virgins. Mean flight time/flight/♀ plotted vs age. Only
JHM-treated ♀♀ had migratory flights

LONG FLIGHT ACTIVITY
FLIGHT TIMES of VIRGIN FEMALES

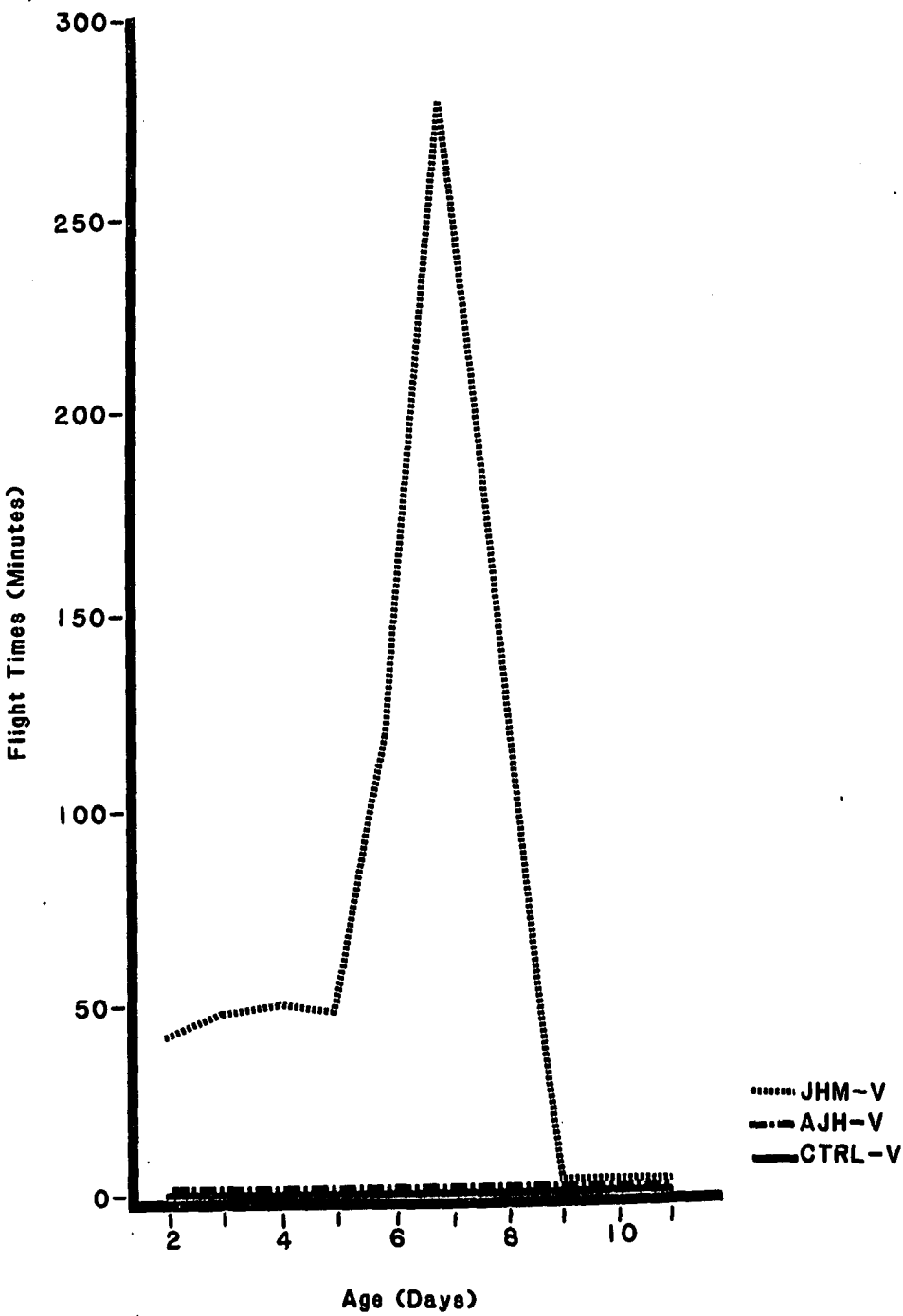
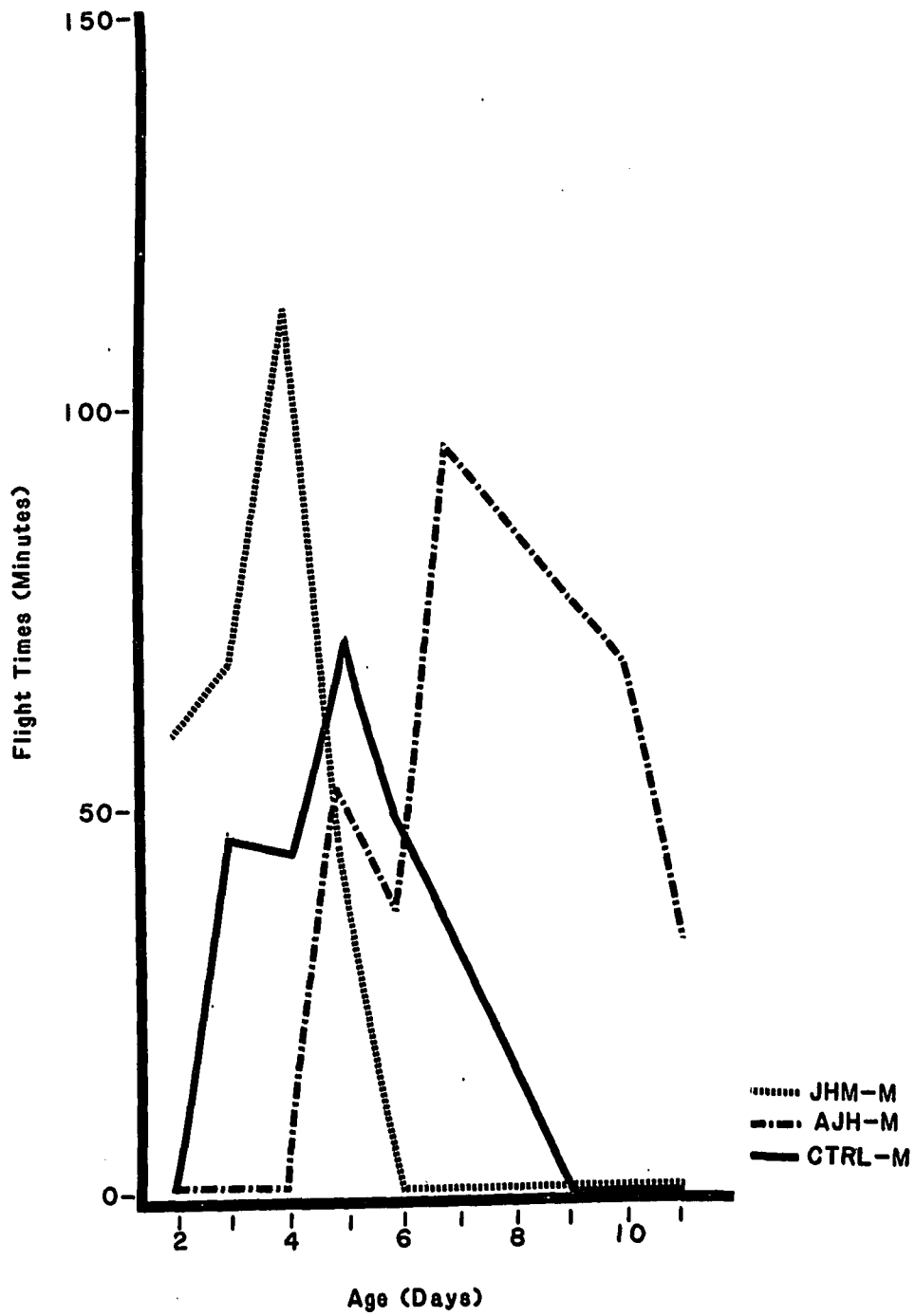


Fig. 10. Combined analysis for long flight activity for all mated ♀♀. Mean flight time/flight/♀ plotted vs age. Note the major age displacement effect for both the JHM and AJH treatments, as well as the higher levels of activity when compared to the control

LONG FLIGHT ACTIVITY
FLIGHT TIMES of MATED FEMALES



SUMMARY

One purpose of this research was to assess the potential for migratory flight in the WCR. A second objective was to determine how flight activity is regulated by such factors as JH, photoperiod, and nutrition. Finally, I suggest that ovarian development is correlated with flight duration and controlled by application of juvenile hormone mimic (JHM) or juvenile hormone inhibitor (AJH).

There is circumstantial evidence that female beetles have considerable flight ability, but quantitative data are lacking. Quantitative flight data might explain the rapid reinfestation of first year corn, and also illustrate the means by which WCR expanded its range from the Rocky Mountains to the eastern seaboard in forty years. With a flight mill interfaced to a microcomputer, we determined that there were two types of flights, trivial and sustained. Trivial flights lasted a few minutes, and were one to ten meters long. Sustained flights lasted an average of 72 minutes, with a mean distance of over 3600 meters. Long flights are considered to represent aseasonal migration. They occur most often in five- and six-day-old preovipositional females.

Once flight activity patterns had been established, the second objective was examination of possible catalysts of flight activity. Control of the dispersal of this insect is necessary to limit damage to corn. One means by which management of the WCR may be achieved, is the disruption of the biological mechanisms effecting flight activity. An understanding of the flight catalysts is necessary before it is possible to disrupt these processes.

Since field-collected adults and adults reared from eggs in the laboratory exhibited similar flight behavior, the environmental cues of photoperiod and nutrition were not considered to be catalysts. Because the level of JH had been shown to significantly affect flight activity in other Coleoptera, alteration in the levels of JH was considered as a more probable mechanism. Levels of JH were changed through topical application of JHM and AJH. Use of mated and virgin females permitted an evaluation of the JH effects on flight normally found in females. These normal effects then were compared to the effects found when JH levels were modified. The results of these treatments were significant. The effect of JH on trivial and migratory flight activity in this species was determined.

In the first part of this research, complete ovarian dissections were made for females that had flown migratory flights. Only mating status was confirmed for trivial fliers. The results indicate that there is a minimal ovarian development in beetles nine to eleven days old. Sustained fliers were mated, but they had only preovipositional ovaries characterized by the presence of oöcytes in the vitellarium, with yolk deposition in progress.

For the second half of this research, ovaries in females from different treatment groups were evaluated, using a more detailed breakdown of ovarian stages. The results permitted analysis of the ovarian development in relation to the different JH levels present at the different ages of females. The relationship between female flight and reproduction could then be determined through the combination of

ovarial development and flight activity at different ages within each treatment group.

The degree of ovarian development consistently corresponded to the type of treatment and the amount of flight activity. The use of JHM caused premature ovarian development and flight activity in virgin females. The AJH retarded egg production, and also delayed the age at which peak migratory flight occurred.

Migratory flight activity occurred in JHM treated virgins, with a corresponding maturation of oöcytes, while neither sustained flights nor ovarian development was observed in the controls. AJH totally inhibited all flight activity in virgins. However, developmental differences in the ovaries were not distinguishable with AJH treatment because both treated and control virgins had only undifferentiated cells in the germarium (stage one ovaries).

The most interesting aspects of this research relate to the questions raised by the results. What are the mechanisms by which JH levels affect flight activity? Is there a feedback from the ovaries which regulate JH synthesis in the corpora allata of this species? And are there additional effects from topical application of JHM or AJH, beyond changes in juvenile hormone levels in the hemolymph and ovarian development, which control the flight behavior in this species? Clearly, the increased level of flight activity in treated females over controls raises such a question. Future research in this area will be necessary to provide the answers.

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APPENDIX A:

SCANNING ELECTRON MICROGRAPHS

Plate I. Head of ♂ WCR. Chemically fixed with glutaraldehyde and paraformaldehyde,
post-fixed with OsO_4 . Critical point dried, sputter coated with AuPd. Magnified
100X. Bar = 25.0 μ



Plate II. Head of ♀ WCR. Same preparation as for Plate I. Magnification of 94X. Bar = 28.4μ



Plate III. Ovary of freeze-fractured abdomen of ♀ WCR. Chemically fixed as for Plate I.

Freeze fractured in liquid nitrogen prior to critical point drying and sputter coating. Note the exochorion (arrow), which is distinctive for this species, and easily observed on mature eggs (see Plates IV and V). Magnified 200X. Bar = 62.4μ

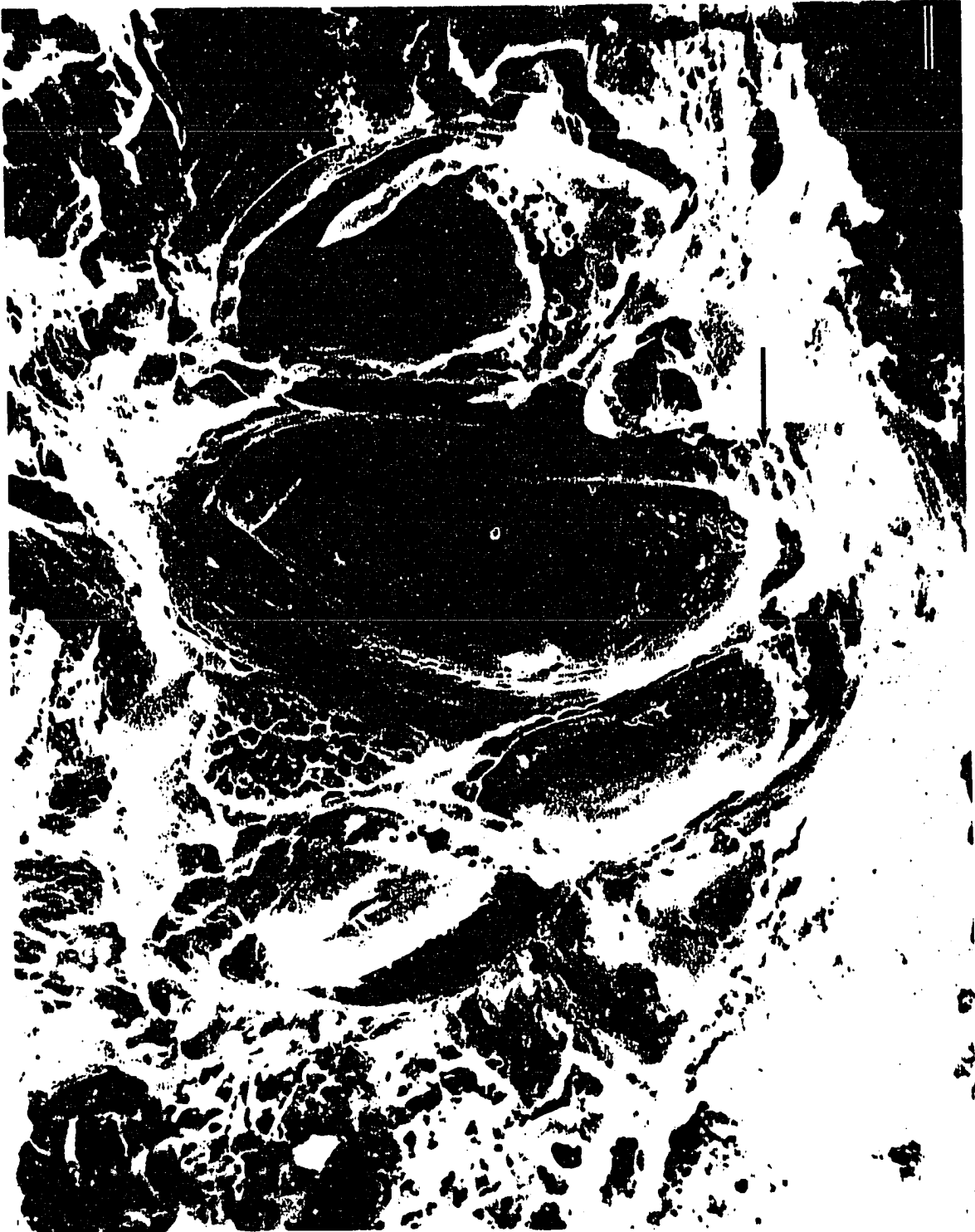


Plate IV. Completely developed egg of WCR. Fresh specimen, only
sputter coated with AuPd. Magnification of 120X. Bar =
55.9 μ

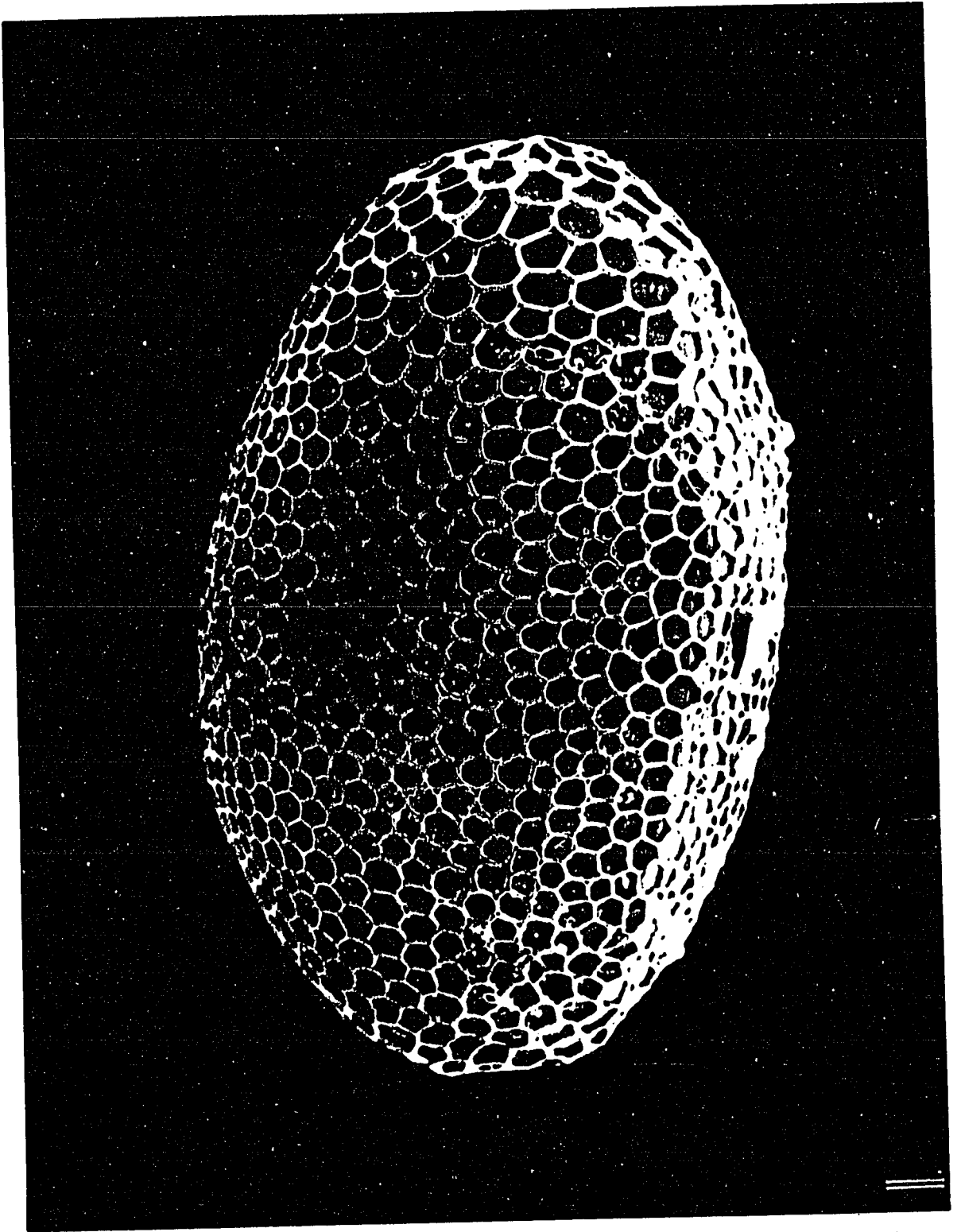
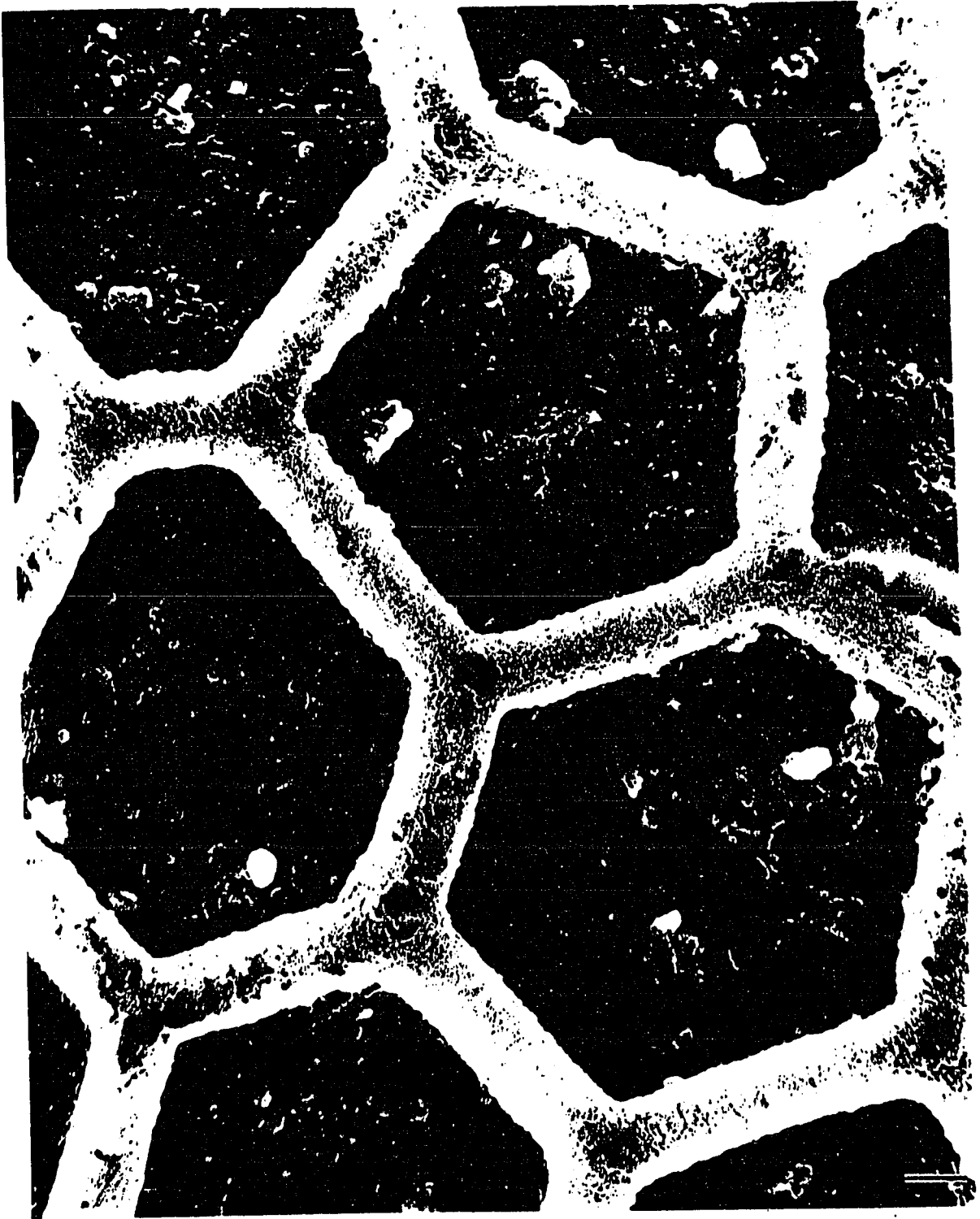


Plate V. Lattice of exochorion. Same WCR egg as in Plate IV.

Magnification of 3000X. Bar = 3.3μ



APPENDIX B:

PHOTOGRAPHS OF FLIGHT MILL EQUIPMENT AND FACILITIES

Plate I. Mounting stage. Microscope with vacuum set-up to hold insect in place while attaching beetle to the flight mill arm

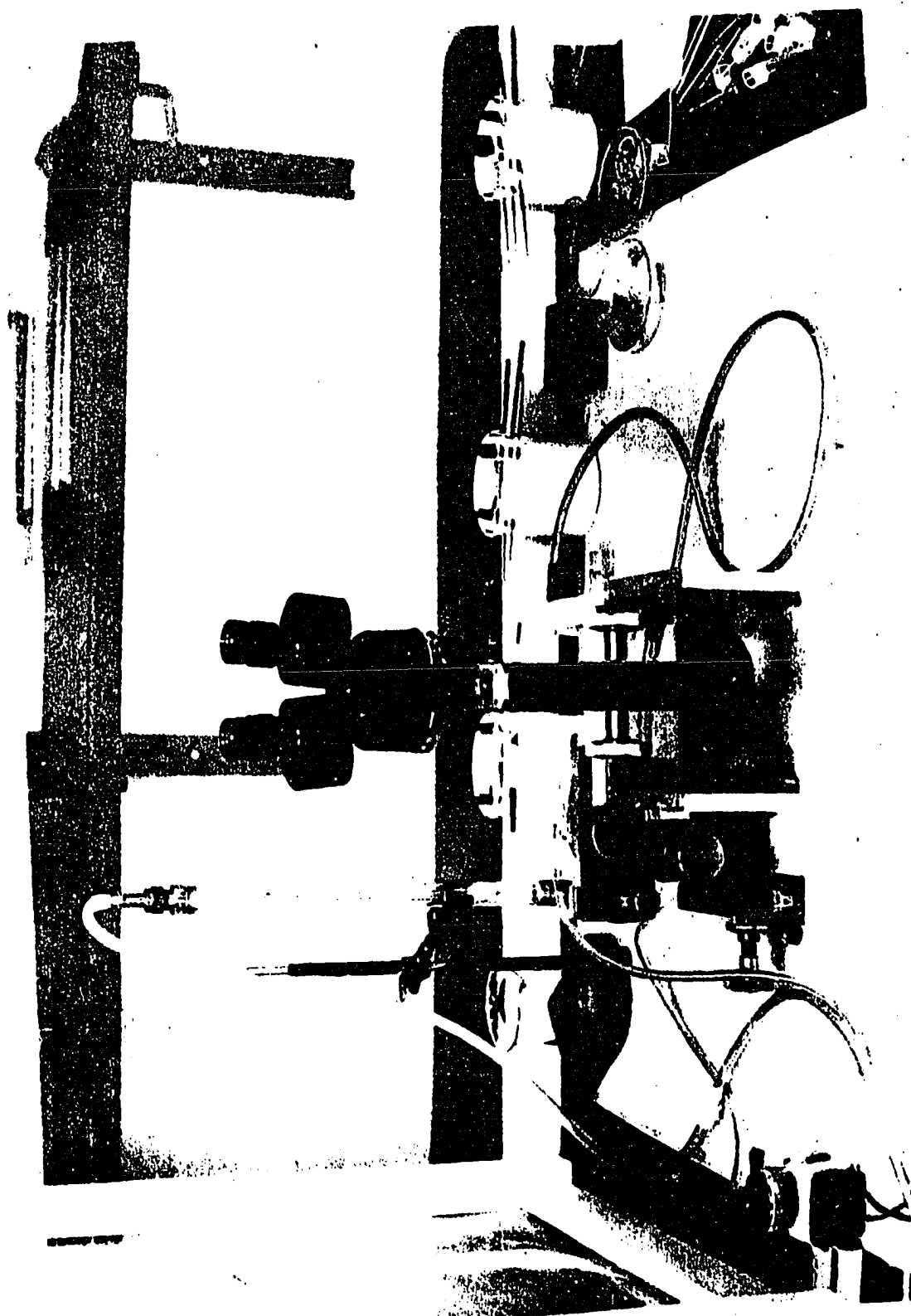


Plate II. Flight mill. Hooded flight mill, on which arm, with beetle attached, is placed

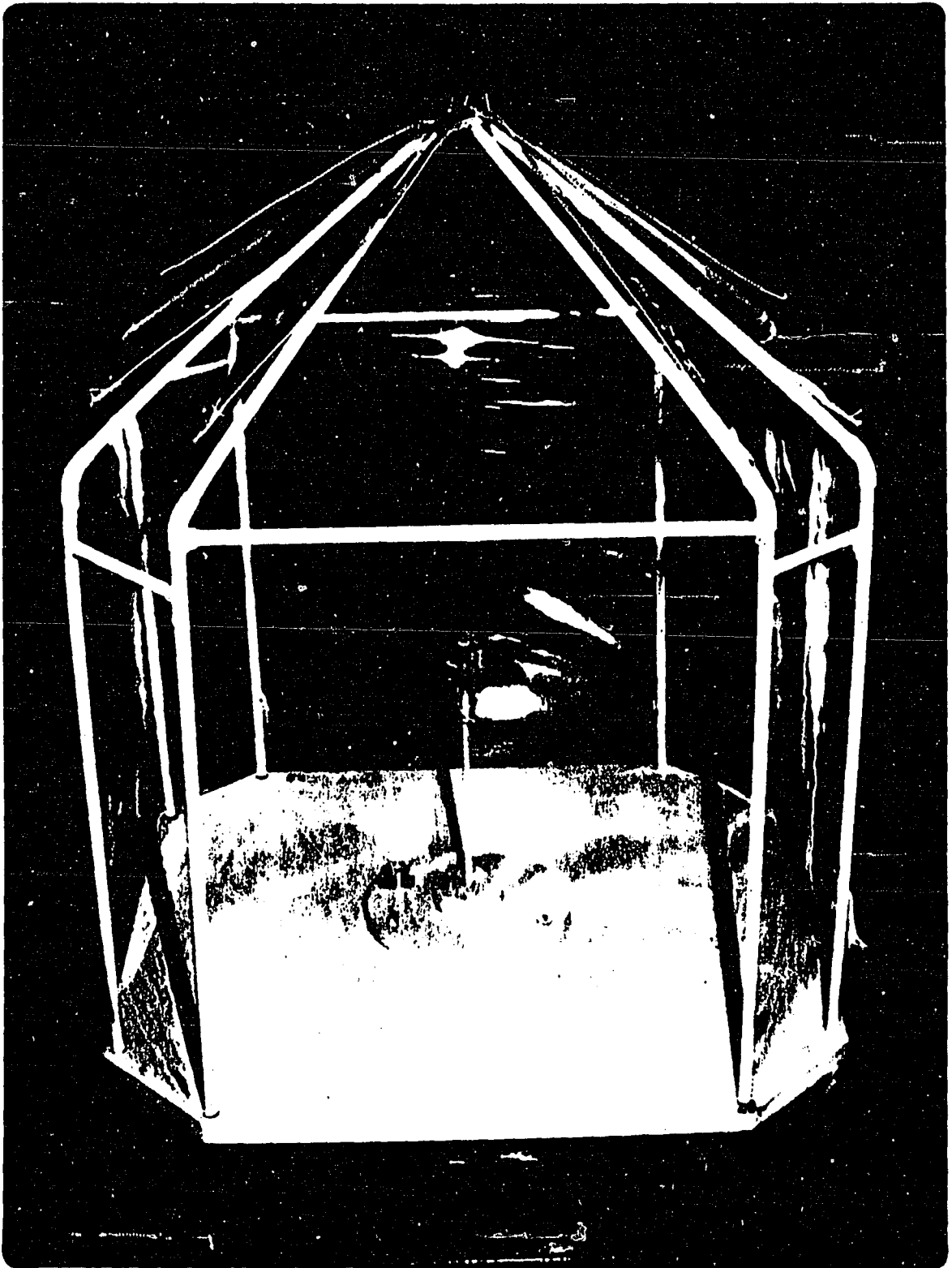


Plate III. Flight mill chamber. Chamber in which twelve flight mills were placed and electronically attached to microcomputer in Plate IV. Chamber was maintained at $25.6 \pm 5^{\circ}\text{C}$ and $80 \pm 5\%$ R. H. A 28-v lamp on each of the twelve flight-mill arms provided the only light

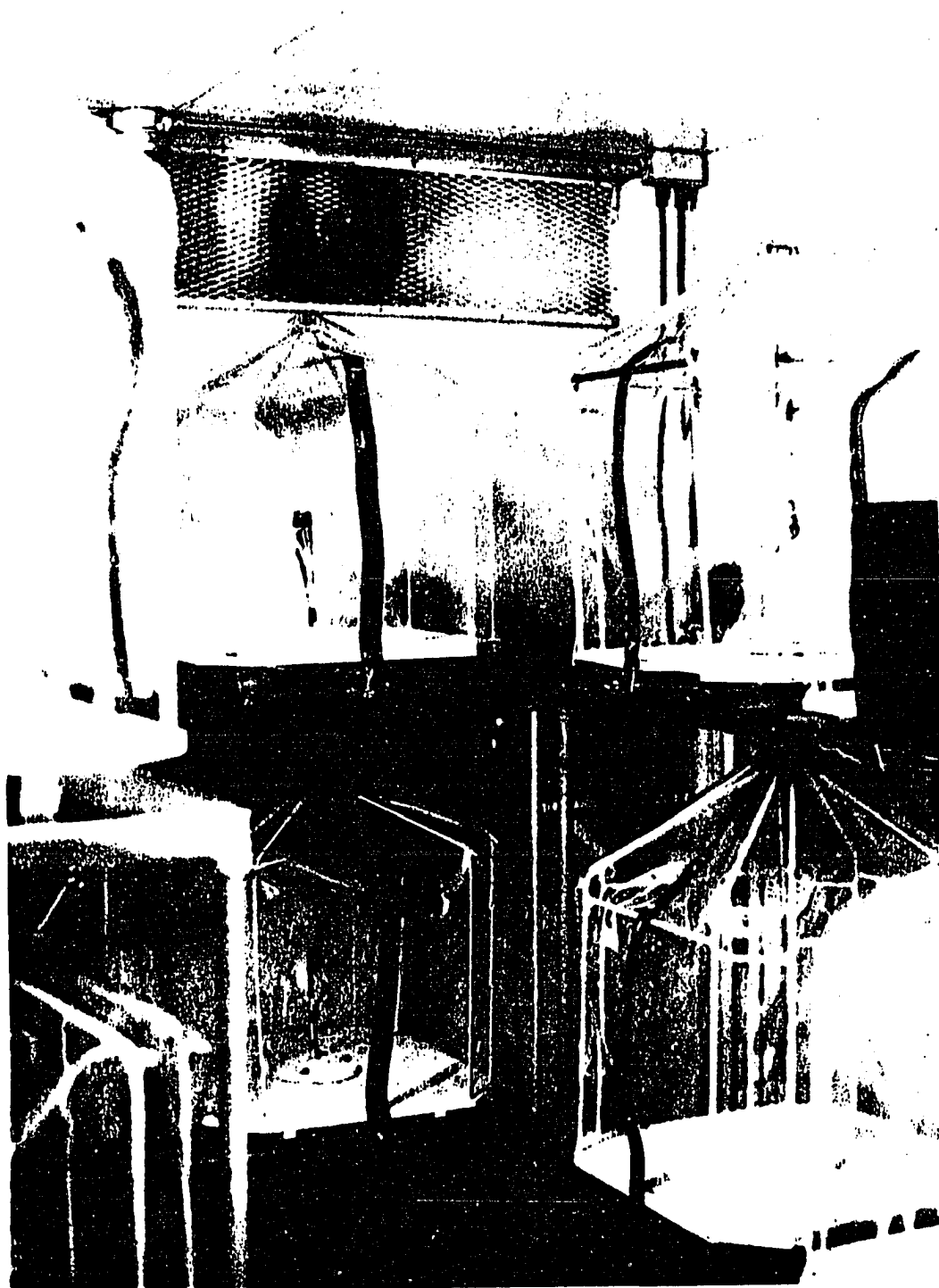


Plate IV. Microcomputer set-up. Microcomputer (Pet Commodore 2400), 2000 Pet-series disk drive and printer are shown with a hooded flight-mill. Electronic signals, generated when the arm passes over the photo cell on each flight mill, were recorded for each mill by the microcomputer. This generated the output of flight duration, distance, speed, and time of day for each flight

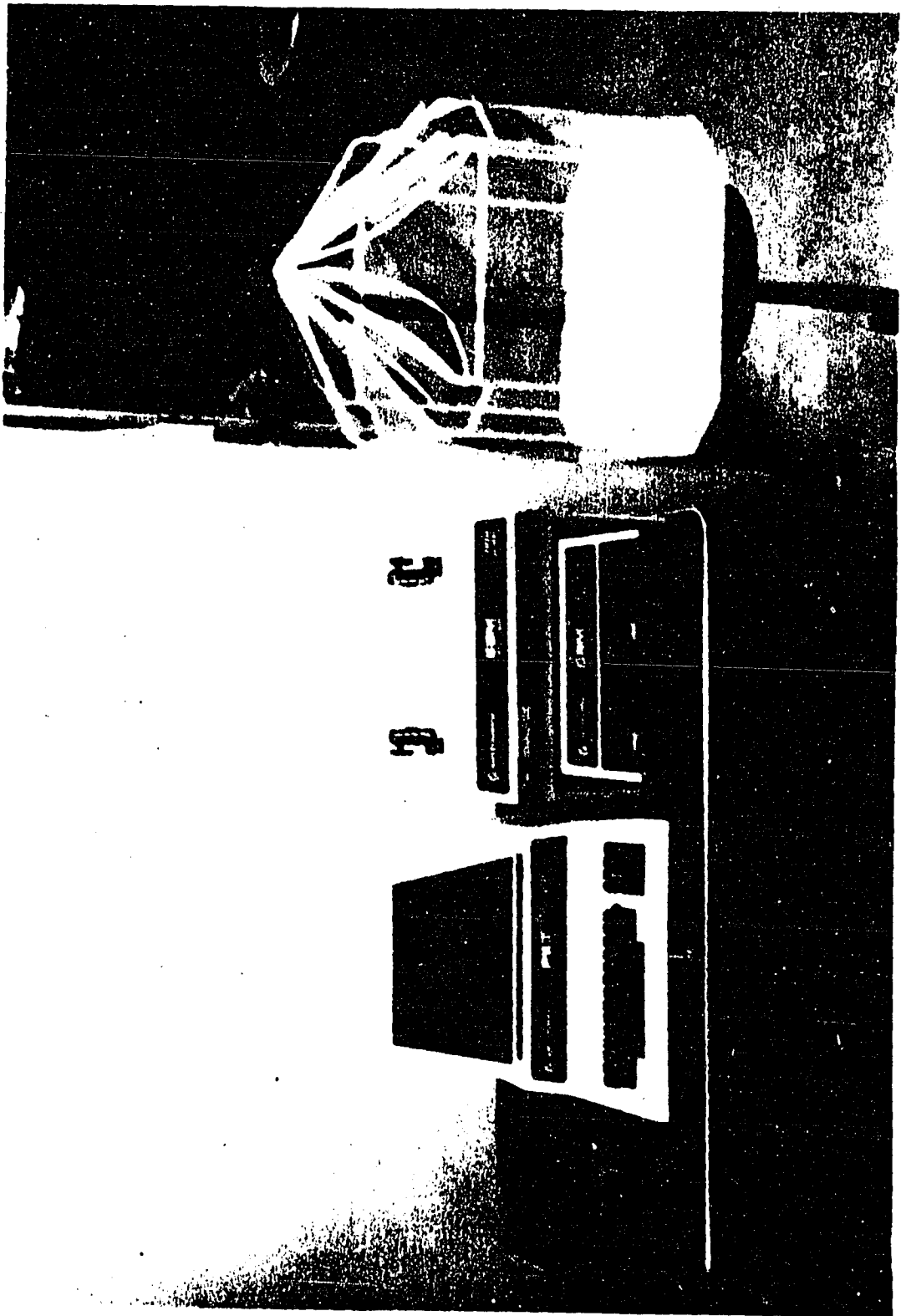
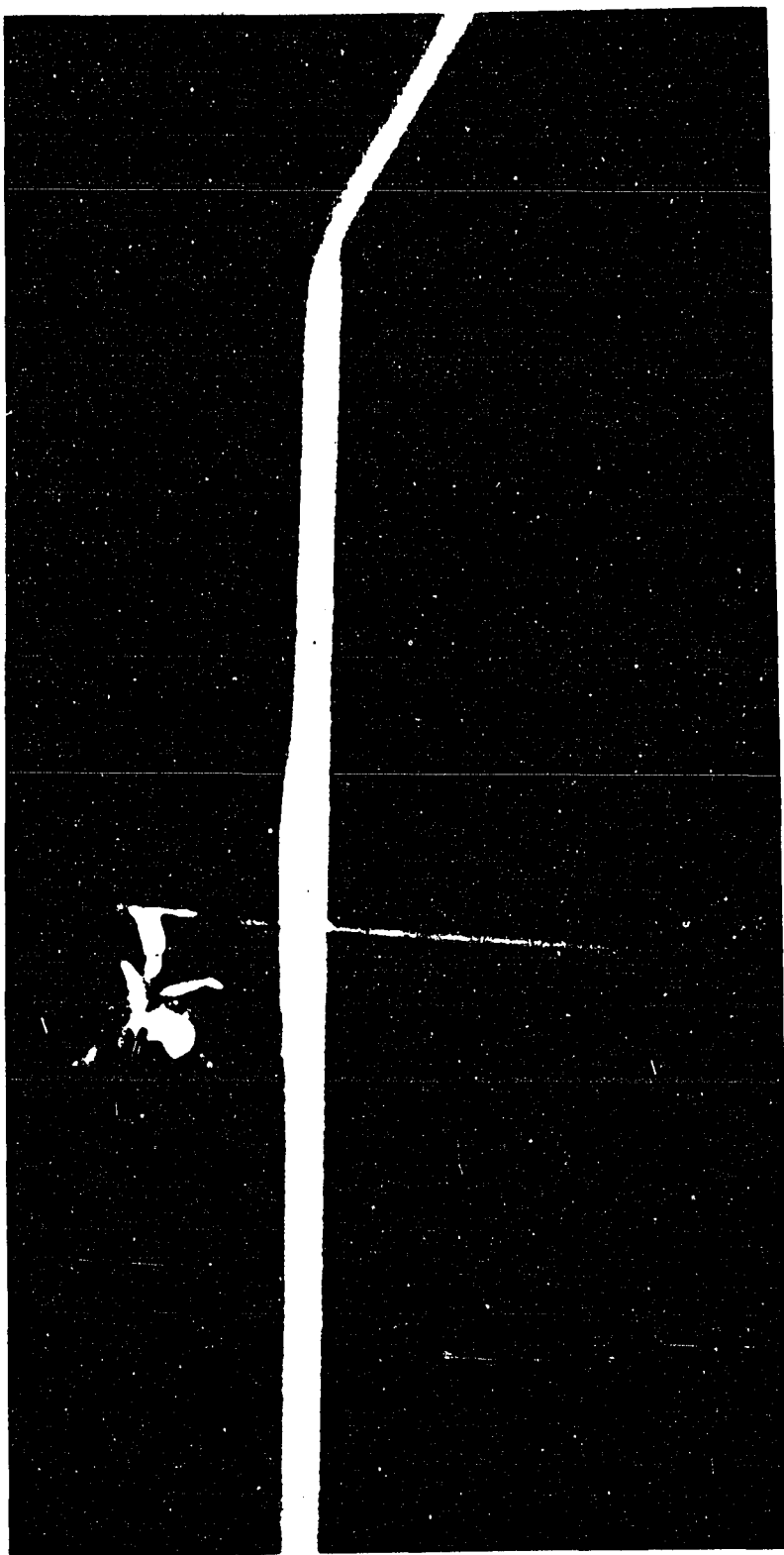


Plate V. WCR ♀ flying on flight mill arm



APPENDIX C:

PHOTOGRAPHS OF REARING EQUIPMENT AND FACILITIES

Plate I. Field emergence screened tent. Daily collection of WCR beetles was made possible by the erection of these 183 cm³ screened cages in cornfields. Beetles were collected for a six week period during the summers of 1983 and 1984

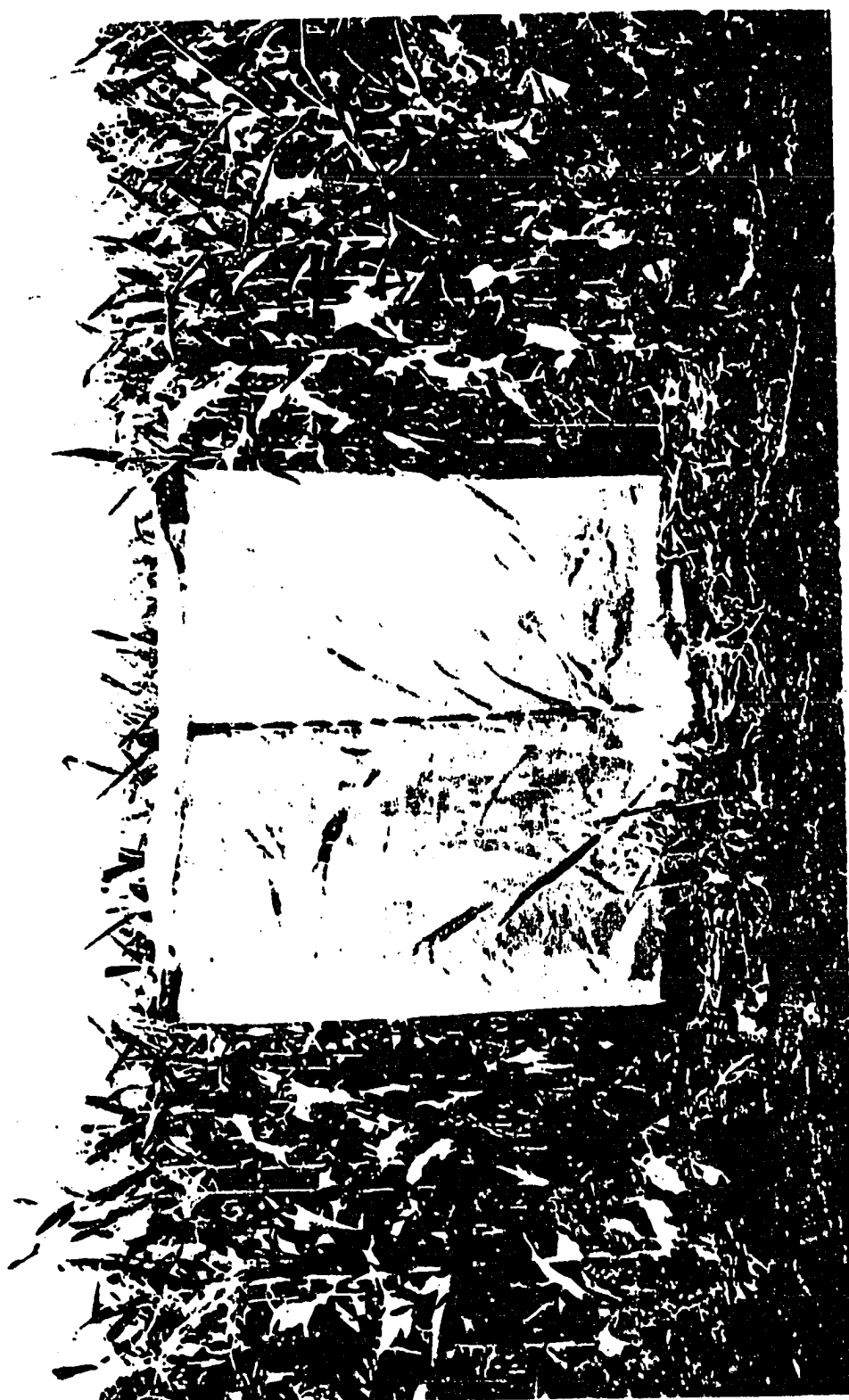


Plate II. Greenhouse cages. Adult beetles were placed in these 30.5 cm³ screened cages in the greenhouse after collection. Each cage contained beetles of the same age. Natural conditions of photoperiod and temperature, and both natural and artificial diet were provided



Plate III. Laboratory rearing of WCR larvae. Larvae were reared in pans of corn placed in screened cages in a rearing room maintained at $25 \pm 5^{\circ}\text{C}$, and $80 \pm 5\%$ R. H. Fresh corn roots were supplied every seven days. Pupation occurred in pans of sterile soil passed through a 20-mesh screen sieve

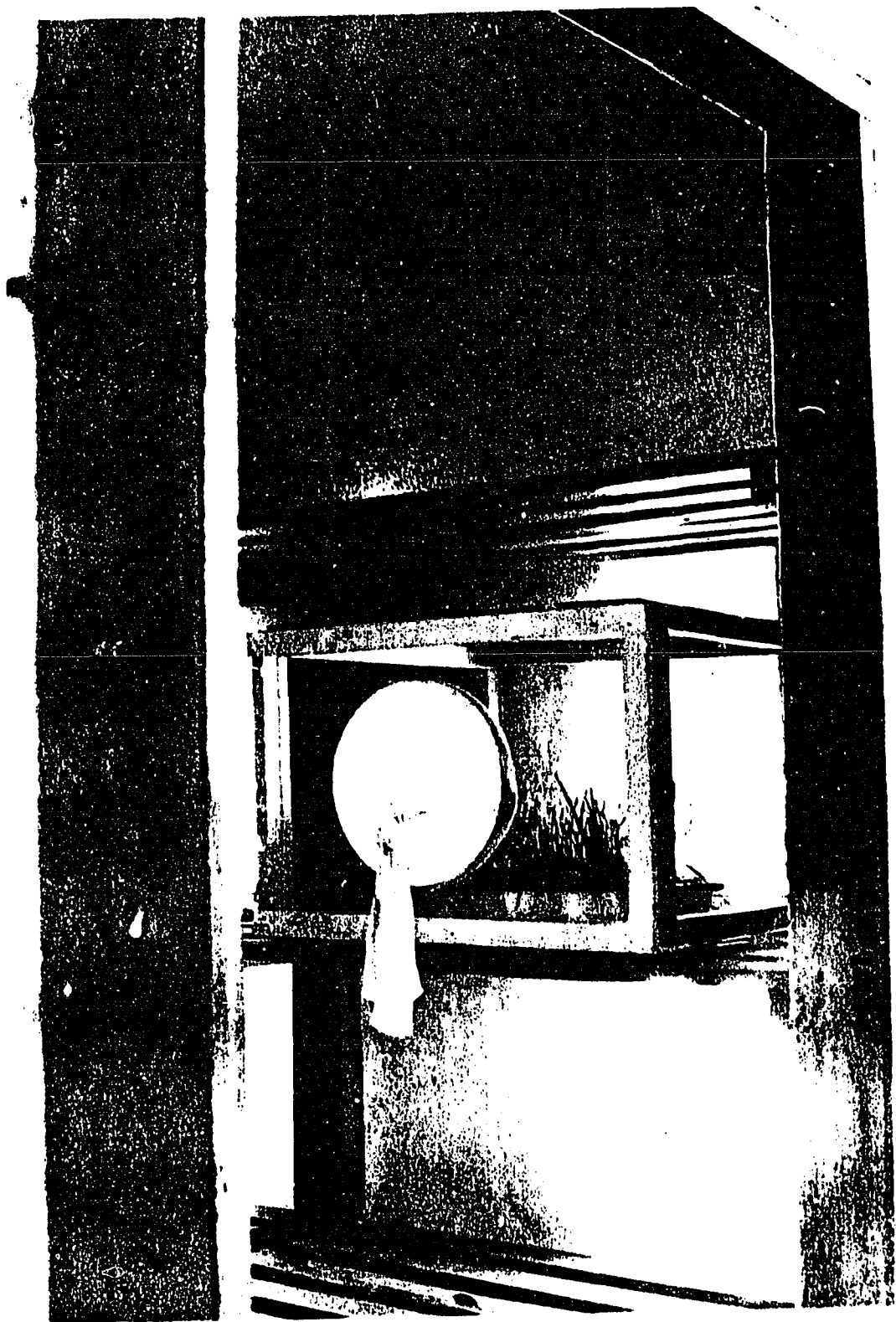


Plate IV. Individual pupation chambers. Rearing of virgins was accomplished by placing late third instar larvae into individual 2.5 cm³ pupal chambers. After emerging, beetles were placed in the greenhouse cages described in Plate II

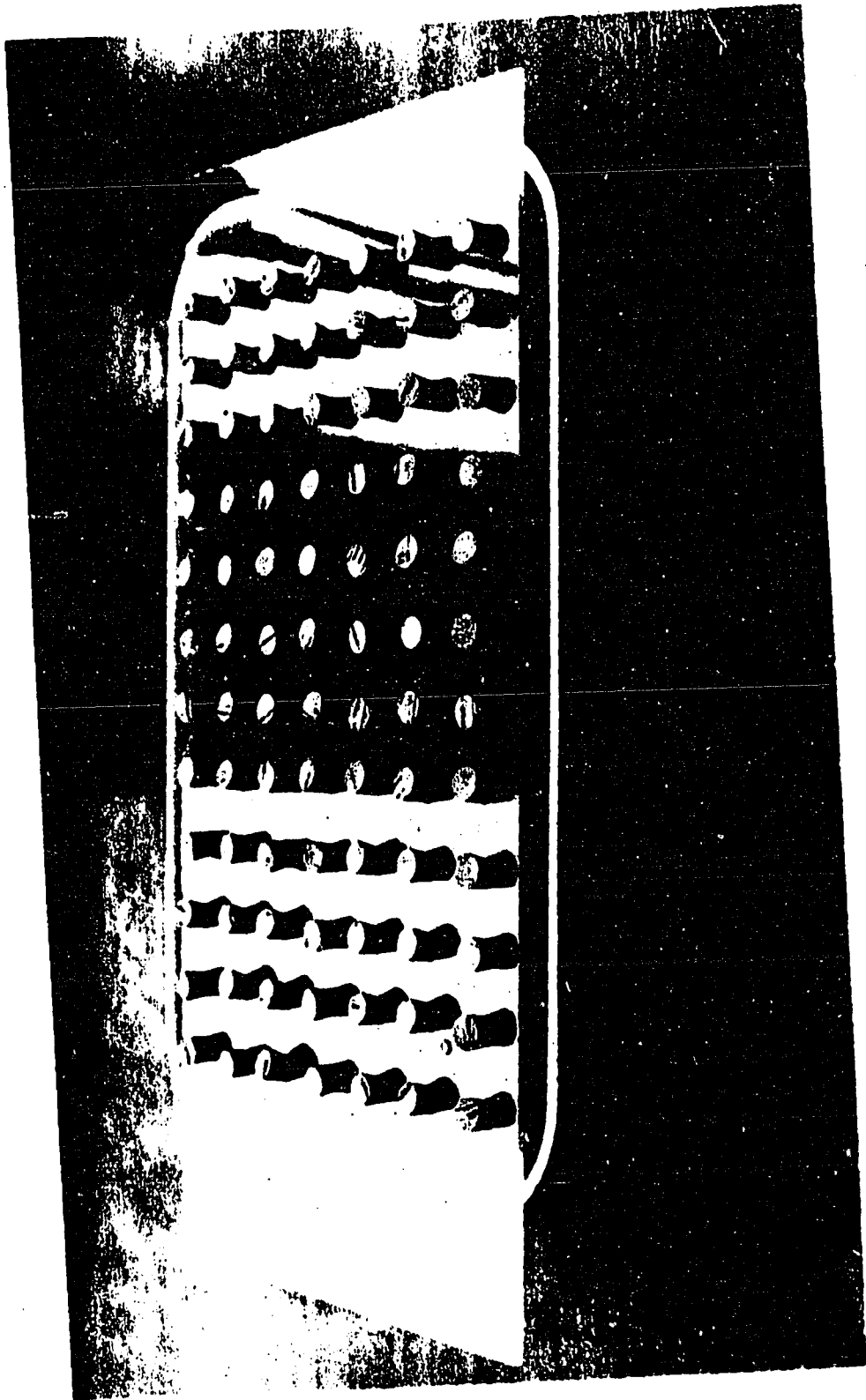


Plate V. Adult WCR on ear of corn



APPENDIX D:

PHOTOGRAPHS OF ♀ WCR REPRODUCTION SYSTEM

Plate I. Dorsal view of ♀ abdomen. Note the non-division of the terminal sclerite. The ♂ has this sclerite divided. This characteristic is used to distinguish the sexes of this species. Bar = 50.8 μ



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Plate II. Reproductive system of ♀. O = ovary, A = accessory gland, C = common oviduct,
S = spermatheca, Sgl = spermathecal gland, V = vagina, BC = bursa copulatrix,
Ov = ovipositor. Bar = 10.3 μ

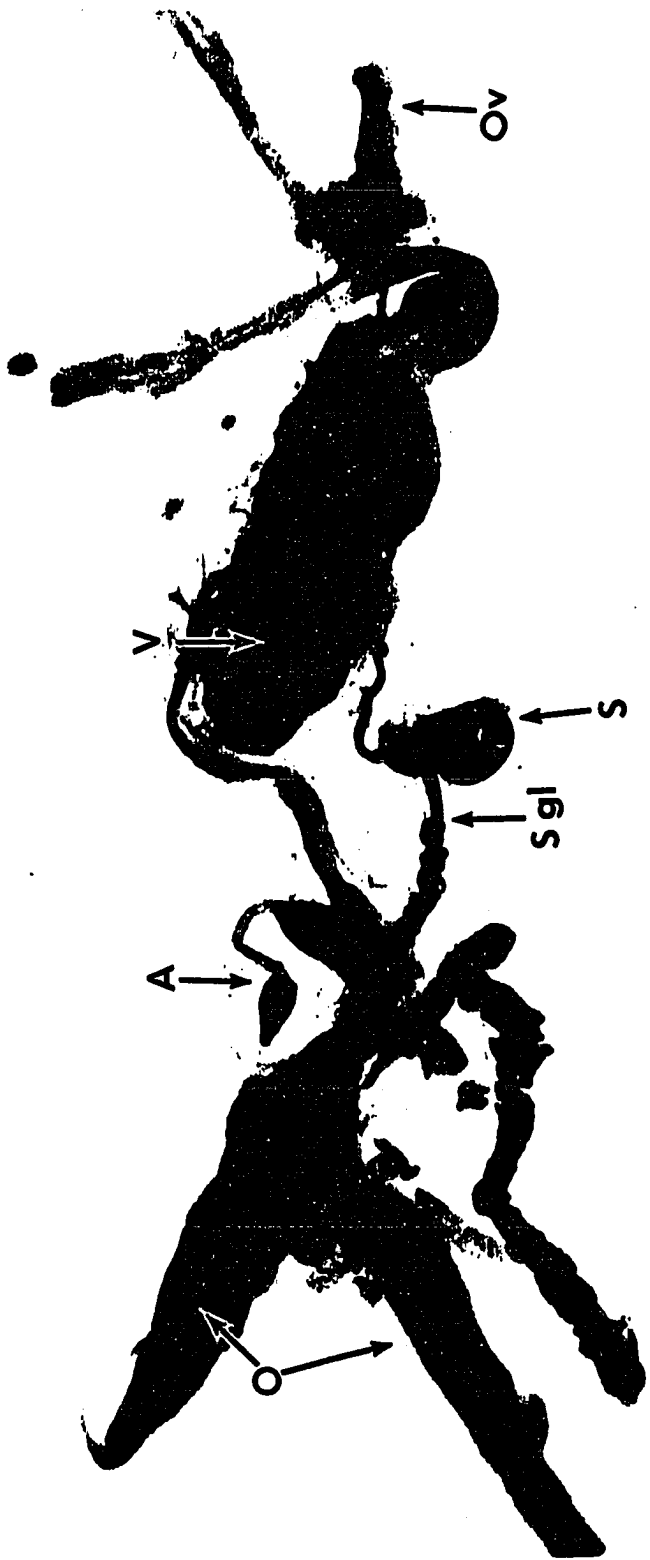


Plate III. Ovipositor of ♀. Bar = 52.1 μ

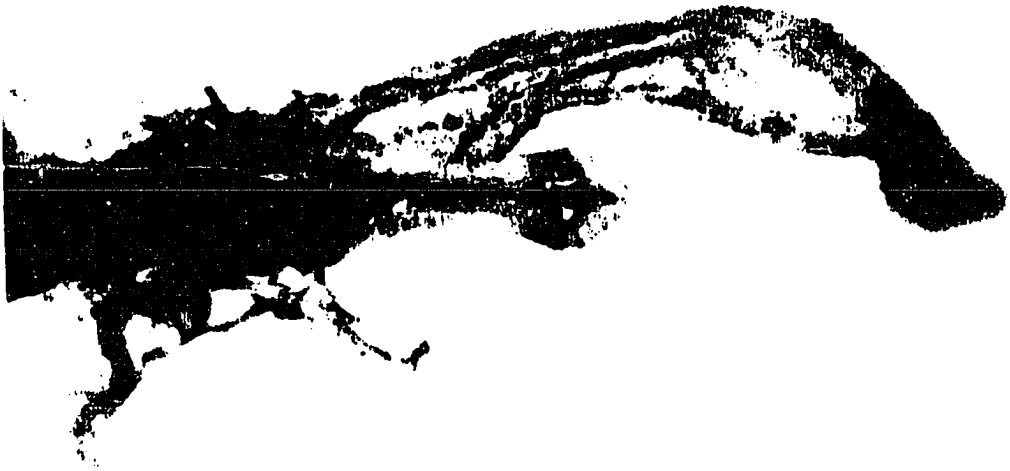


Plate IV. Enlarged view of spermatheca (S) illustrating sclerotization and attachment to spermathecal gland (Sgl). Bar = 62.5 μ

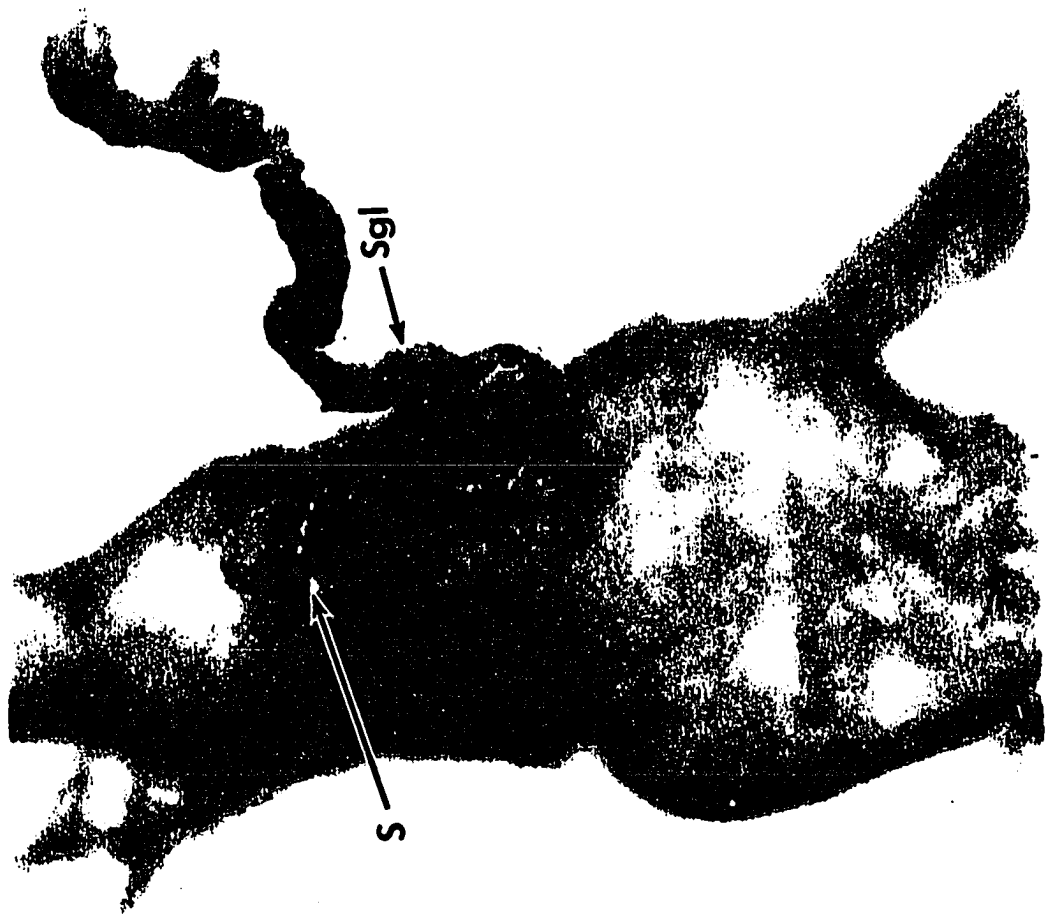


Plate V. Underdeveloped ovary representative of virgin ♀♀ or mated ♀♀ less than 4-days-old. Note median filament (MF) that attaches ovary to body wall.

Bar = 31.3 μ



Plate VI. Mature ovary of mated ♀ 14-days-old. Note germarium (G) where cell differentiation occurs, and vitellarium (V) where yolk deposition occurs.

Bar = 95.4 μ

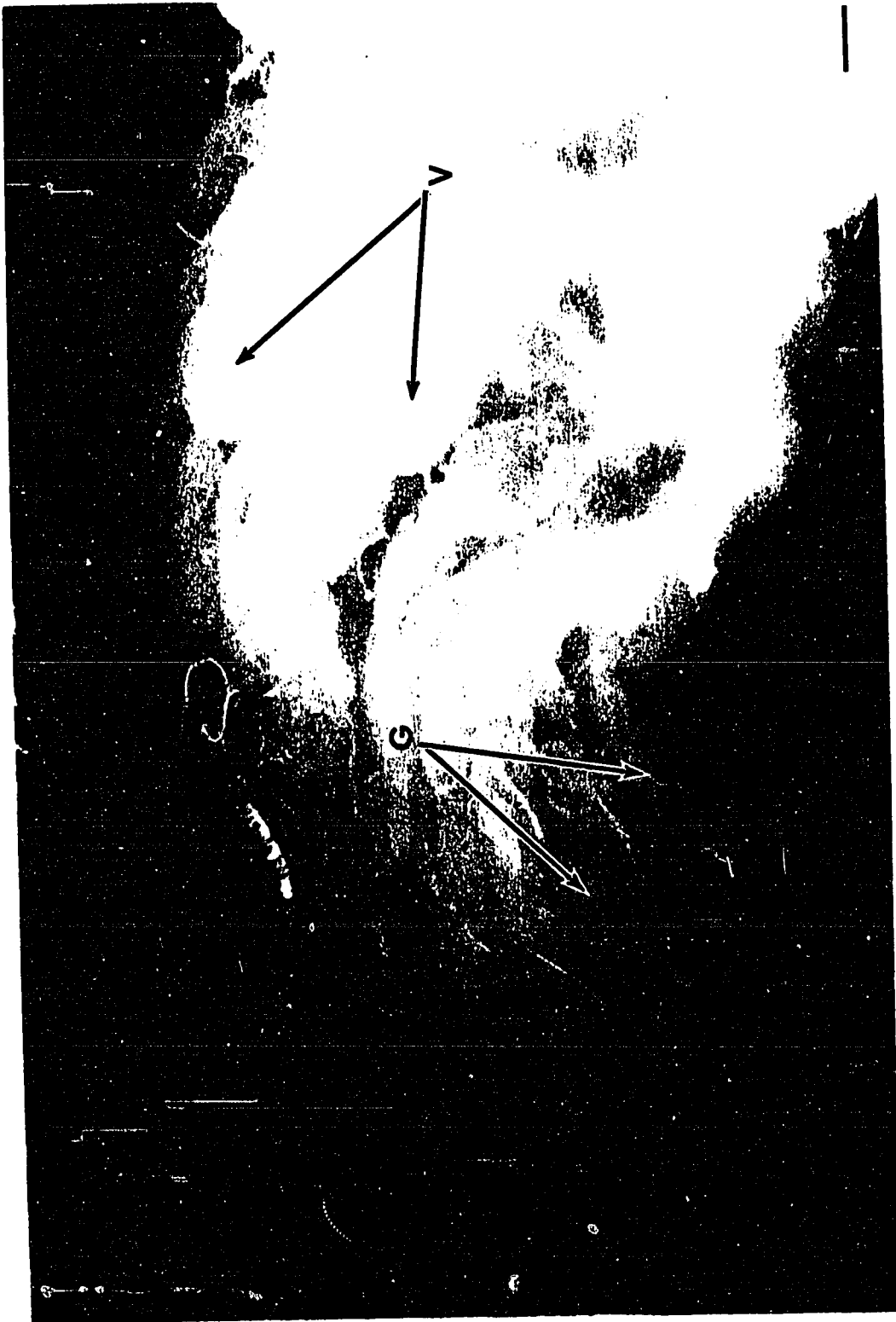


Plate VII. Mature ovary of 18-day-old ♀. Note equivalent development of follicles in neighboring ovarioles (No. 1 - 6). This corresponds to the pattern of laying eggs in clutches observed in the WCR. Bar = 93.8 μ

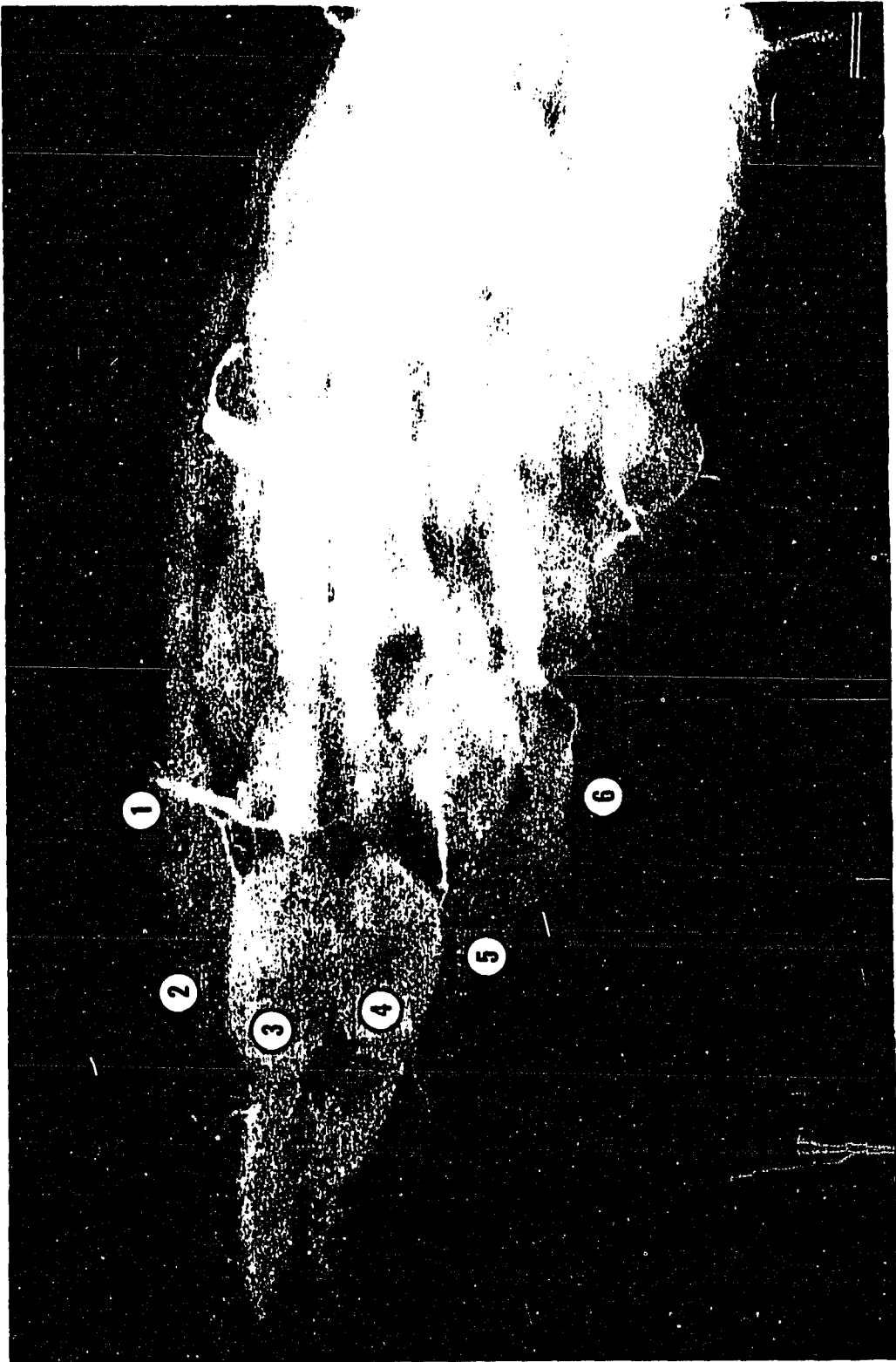


Plate VIII. Mature ovary of 20-day-old ♀. View shows egg calyx (EC), mature oöcytes (Oö),
and lateral oviduct (LO). Bar = 88.9 μ

